

# Haematological modifications in counterpulsation

## Description of a technique for late controls of blood

by

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An increase in hemolysis after mechanical trauma of the blood was first recorded in 1884 by Meltzer and Welch<sup>14</sup> who wrote: "If the blood shaken... for a period so short that no visible change in the blood has occurred, and then the blood be allowed to stand at rest, it will be found that at the end of 15 to 18 hours, nearly all the red blood corpuscles have completely lost their colouring matter, while the corpuscles in the control specimen are still wholly intact".

The mechanical circulatory supports demand thoughtful consideration of the continued consequences of the trauma to blood by the extracorporeal circulation. Immediate hemolysis has been the commonly accepted index of trauma to blood and has usually remained well within the limits of physiological tolerance.

As Bernstein and co-workers pointed out,<sup>3</sup> severe postperfusion anemia accompanied by a markedly shortened red blood cell life span, suggests that immediate hemolysis may represent but a small fraction of those red cells seriously damaged during pumping. They are indeed able to induce hemorrhagic and/or intravascular clotting syndrome.

The present study was performed to characterize and quantitate the manifestations of haematological modifications during and following cardiac assistance by counterpulsation in the dogs.

First, we shall describe an improved technique for repeated withdrawals of blood samples.

## METHODS

Haematological values and particularly life span studies require frequent blood samples during several days. This is a real problem in the dog because repeated venipunctures are more and more difficult and on the other hand, the animal becomes suspicious and shrinks away from the needle.

Its movements may alter the quality of blood samples especially when haematological studies are performed. We have therefore devised a technique of long term plastic tubing implantation into the jugular vein as follows. The dog's external jugular vein<sup>3</sup> unlike that of man, is the main channel for return of venous blood from the head. It is about 1 cm in diameter and 12 cm long.

It lies directly under the skin and pulsations are visible through it. Under general anesthesia, a 5 cm longitudinal skin incision is made along the external jugular vein which is exposed. Silastic tubing is introduced into this vein along 15 to 20 cm.\* The other end of the tubing is pulled under the skin of the anterior and lateral part of the neck and pulled out at the back in the dorsal region after a subcutaneous course of approximately 20 cm. The external part of the tubing is sutured on the skin and is closed by a *stop-cock* (Fig. 1). A wide bandage around the neck protects the tubing from being torn out.

\*Silastic medical grade tubing. Dow Corning international Ltd.

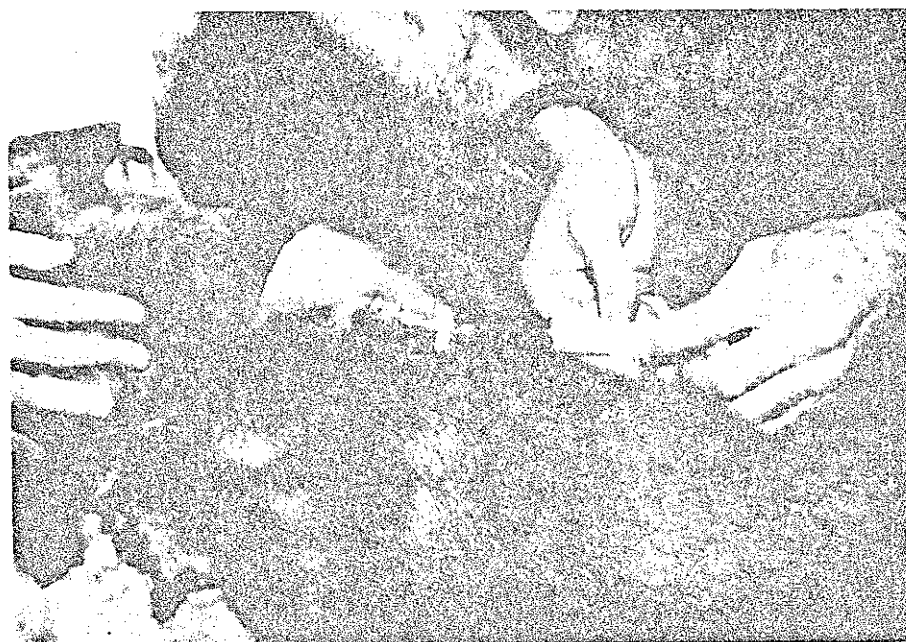


Fig. 1.—Withdrawal of blood sample by the implanted plastic tubing.

In order to avoid clotting of the blood in the catheter, heparinized saline ( $\frac{1}{2}$  mg/kg) is slowly injected, just enough to fill all the tubing. This injection is repeated every day. By this procedure we could perform daily withdrawals of blood during three weeks.

An almost similar technique of long term plastic tubing implantation was recently published by Itoth *et al.*<sup>12</sup> although we have been using ours for a year.

Synchronous cardiac assistance by counterpulsation was effected by a Simas pump. Twenty experiments were conducted on healthy fasting mongrel dogs. Properly synchronized counterpulsation was achieved for two to four hours. The stroke volume of the pump was equal to 50 or 100% of that of the dog. Haematological modifications during and after counterpulsation are studied in this paper. Dogs were submitted to general heparinization at the beginning of the experiment.

Red blood cell damage was assessed by measurement of hemoglobin, hematocrit, plasma hemoglobin, osmotic fragility

and half-time of  $\text{Cr}^{51}$  labelled erythrocytes. Plasma hemoglobin was analysed during counterpulsation by the method of Flink and Watson.<sup>9</sup> Osmotic fragility was based upon the method described by Parpart and co-workers.<sup>16</sup> The radioactive-chromium method was carried out as follows in order to know the immediate and the delayed hemolysis after counterpulsation. Approximately 15 ml of blood are delivered into 5 ml of sterile acid citrate dextrose (ACD) for labelling with approximately 50 to 100  $\mu\text{Ci}$  of  $\text{NaCr}^{51}\text{O}_4$ . Counterpulsation is started immediately after injection of the  $\text{Cr}^{51}$  labelled red cells.

Blood samples for measurement of radioactivity were obtained during the counterpulsation every 15 minutes for one hour and daily after the counterpulsation during 10 to 15 days.

Plasmin activity was investigated by fibrinogen dosage and by Astrup plate technique<sup>1</sup> in some dogs during the counterpulsation.

Quantitation of fibrinogen, platelet count, histological and immunofluorescence study with antiplatelet and anti-

fibrinogen sera were performed in order to visualize widespread *intravascular coagulation*. Antifibrinogen and antiplatelet sera were respectively obtained by a weekly injection of dog fibrinogen and platelets and mixed with Freund's adjuvant to rabbits during 6 weeks. Fluorescent antibodies were prepared by the dialysis technique of Clark and Shepard.<sup>4</sup> Coons and Kaplan's method<sup>5</sup> was used for the detection of fibrinogen and platelets in tissues.

TABLE 1.—Plasma hemoglobin concentration (mg%) during counterpulsation (CP).

Experiment No.	Before CP	1-hr CP	2-hr CP	3-hr CP	4-hr CP
1	6.6	10.1			
2	7.0	12.6			
3	5.1	8.0	20.9		
4	7.1	9.1	6.7	10.6	
5	9.3	8.6	29.0	23.2	20.0

### RESULTS

1. *Red blood cell damage*: immediate red blood cell trauma was studied during 2 to 4 hours of counterpulsation and the delayed effects for a period of 10 to 15 days after the diastolic augmentation. There was no significant alteration in hemoglobin, hematocrit and red blood cell count during counterpulsation.

Moderate but constant decreasing of the osmotic fragility was noted during counterpulsation, remaining however within the range of normal values (Fig. 2). Table 1 summarizes the changes of plasma hemoglobin concentration during counterpulsation in 5 experiments. Increase of plasma hemoglobin was a constant feature but of little value where-as it did not exceed 30 mg%.

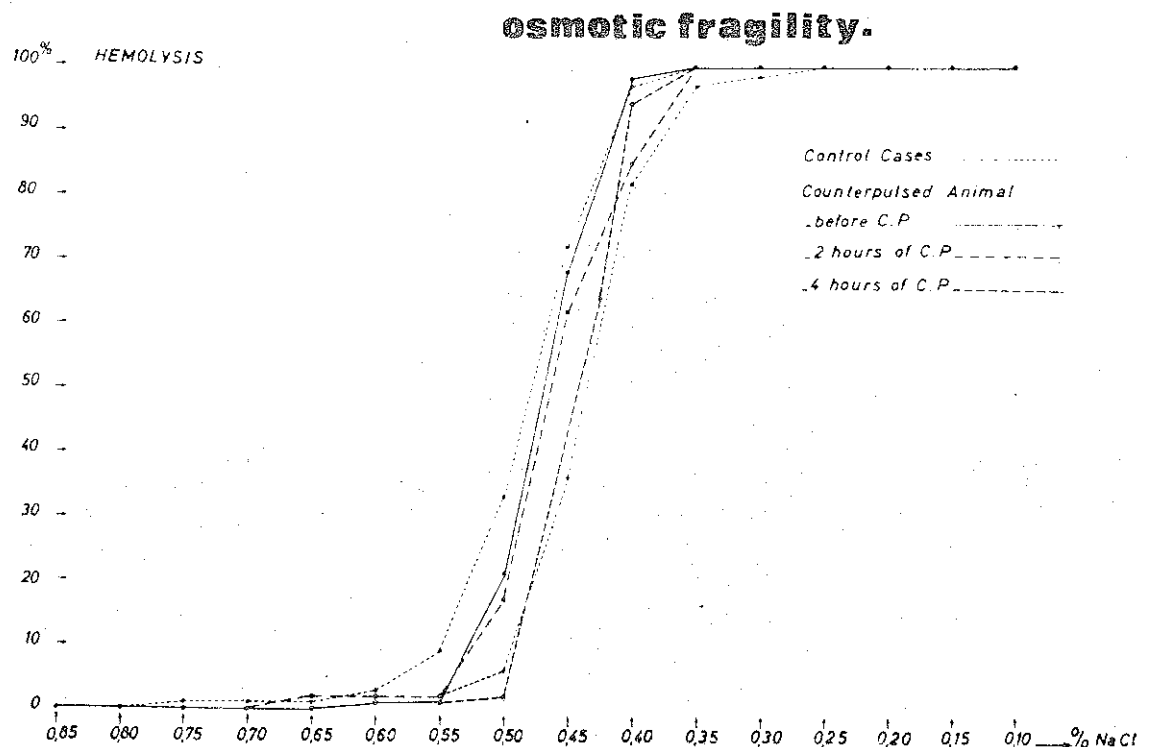


Fig. 2.—Osmotic fragility curves during counterpulsation. The normal range (control cases) is indicated by the dotted lines.

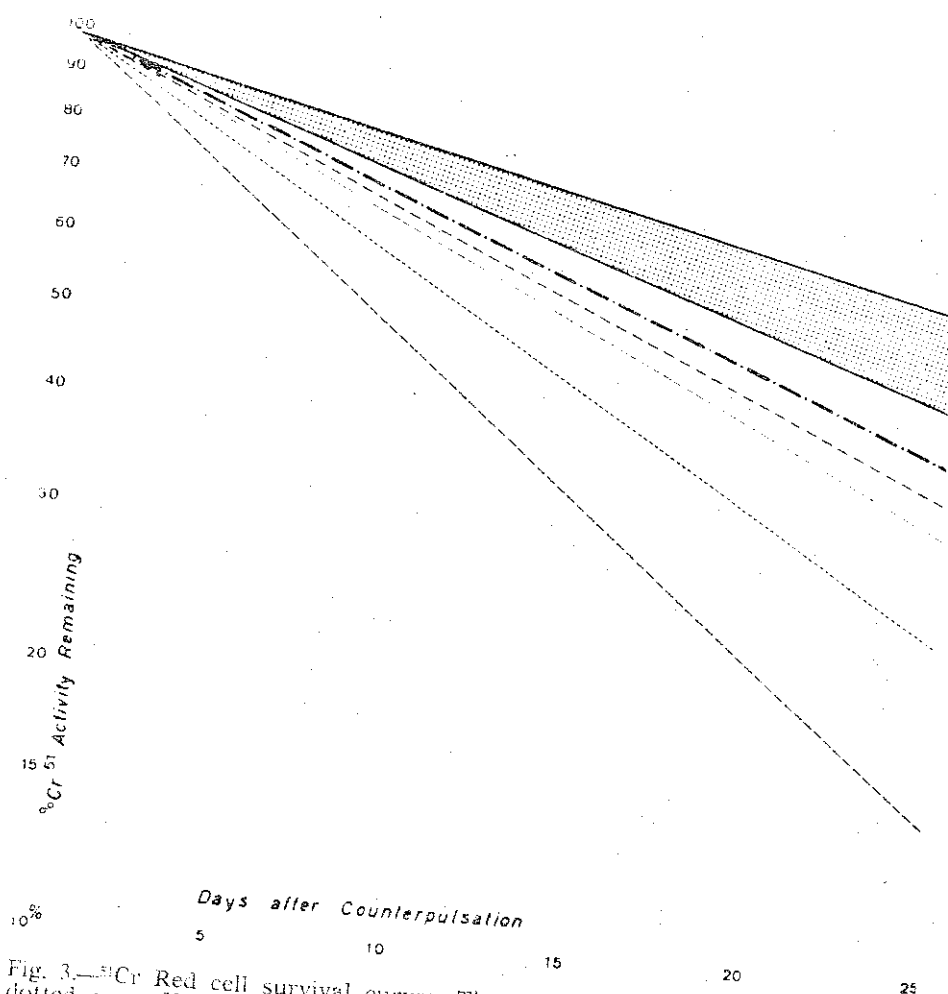


Fig. 3.— $^{51}\text{Cr}$  Red cell survival curves. The normal range is indicated by the dotted area. Half life span of erythrocytes is reduced from one third to one half.

A decrease in hemoglobin and hematocrit to about 20-10% of the normal values was observed in some animals 2 or 3 days after counterpulsation.

More striking features were evidenced by the radioactive chromium method. Postcounterpulsation erythrocytes survival curves, determined with  $\text{Cr}^{51}$ , are plotted in figure 3.

Normal published values of apparent half-time survival of dog erythrocytes (dotted area) vary from 21 to 30 days.<sup>17</sup>

After counterpulsation, there is a more rapid decreasing of the percentage of  $\text{Cr}^{51}$  activity, mean half life span of erythrocytes being reduced from one third to one half of the normal value.

In order to divide immediate and delayed decrease of radioactivity during counterpulsation, the former has been compared with this one occurring in following days.

In figure 4, decreasing of  $\text{Cr}^{51}$  radioactivity is divided into two parts: a sharp slope during the counterpulsation and a slower one for the latter. In the 3 experiments which are summarized in this figure decrease rates of  $\text{Cr}^{51}$  radioactivity during the first hour of counterpulsation are respectively: 17%, 19% and 26% whereas daily decrease of radioactivity after counterpulsation approximates 5 to 7%.

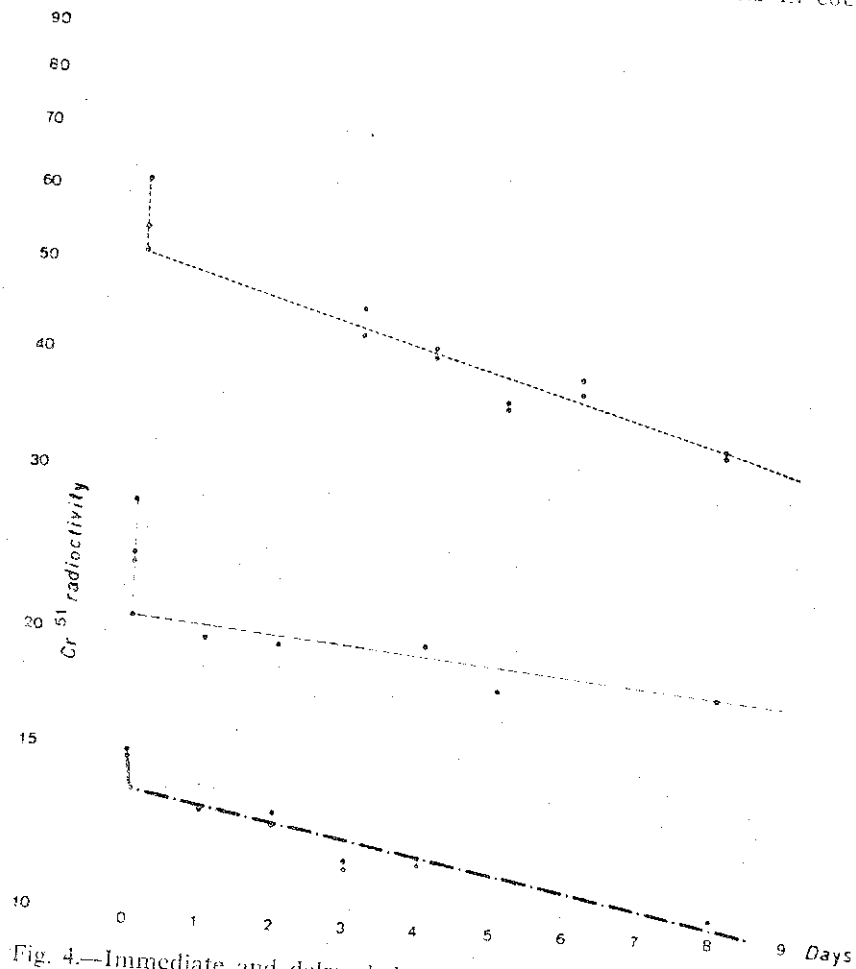


Fig. 4.—Immediate and delayed decrease of  $^{51}\text{Cr}$  radioactivity compared during and after counterpulsation.

2. Changes in fibrinogen concentration and plasmin activity were measured during counterpulsation. Samples for fibrinogen dosage were collected every hour during cardiac assistance. Decrease of fibrinogen concentration reaching 14 to 30% is a constant feature occurring as from the end of the first hour. Plasmin activity (Astrup plate technique) was determined during several experiments. Moderate increasing of plasmin activity was observed in most instances. Maximum activity was noted at the end of the counterpulsation.

3. Platelet count, fibrinogen dosage, microscopic observation and immunofluorescence studies were performed in order to evidence disseminated intravas-

cular clotting syndrome. During counterpulsation, a drop in platelet count ranging down 15 to 50% by the end of diastolic augmentation has been observed. The higher is the drop in plasma fibrinogen level, the higher is the fall in platelet number (Fig. 5). These findings being classic data in the intravascular clotting syndrome, we tried to evidence thrombi production in the capillary network by microscopic and immunofluorescence studies.

Fluorescent antiplatelet and antifibrinogen sera can detect platelet and fibrin thrombi in several tissues. We failed to find similar lesions in heart, lung, kidney, intestine, spleen and liver biopsies after 2 hours of counterpulsation. In hemato-

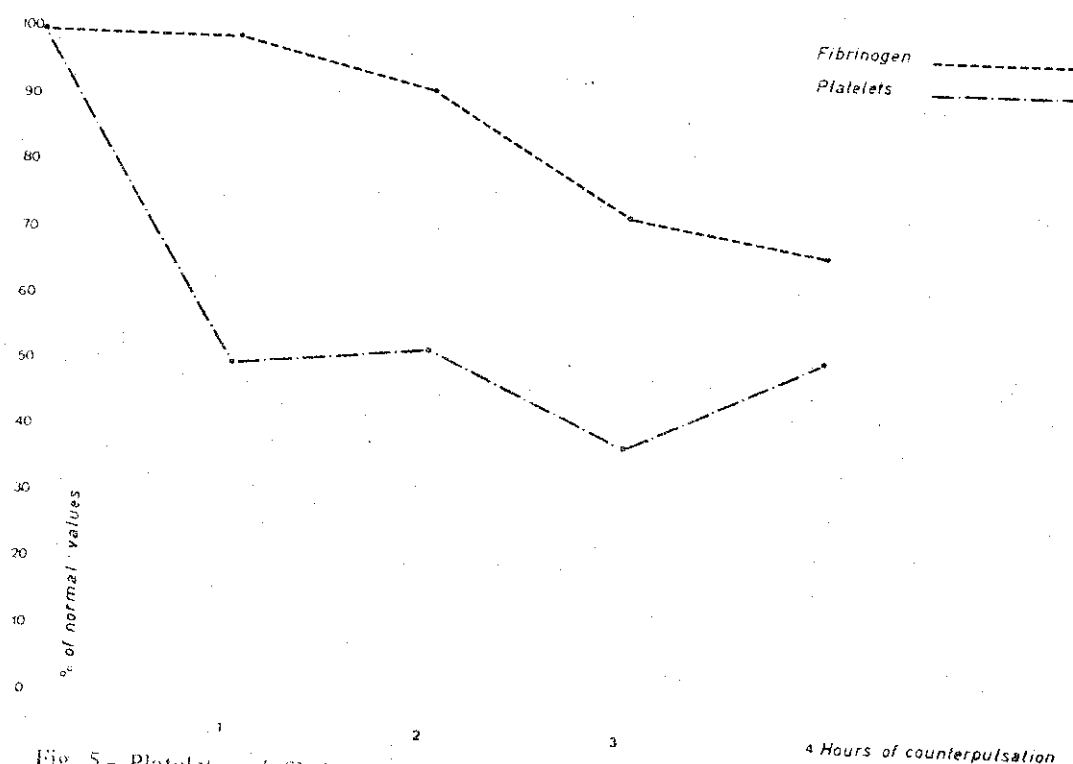


Fig. 5.—Platelet and fibrinogen changes during counterpulsation in one experiment.

xylín-eosin stained tissue sections no platelet or fibrin thrombi were observed, but only capillary dilatations with blood stasis.

#### DISCUSSION

The factors which might be expected to affect the integrity of the red blood cells during extracorporeal pumping have been particularly well studied by Bernstein and co-workers.<sup>2</sup> They include positive and negative pressure pulses, shear forces induced either by local turbulence or by an over-all velocity gradient, interactions between erythrocytes and the surfaces of the pumping system and chemical factors.

In this paper, early and delayed trauma of red blood cells were studied. The concept of sublethal red blood cell damage was first introduced by Galletti<sup>7</sup> and Kuserow<sup>13</sup> who noted the development of profound anemia and shortened RBC life spans in animals that had been perfused

for several hours. Measurements of hematocrit, hemoglobin and red blood cells failed to reveal significant changes associated with counterpulsation. Increasing plasma hemoglobin concentrations remain within well tolerated ranges.

It is interesting to note a decrease of the osmotic fragility during counterpulsation. This is, in fact, a constant feature after prolonged pumping, but it was not described *during* cardiac assistance. Such alterations in osmotic fragility seem actually to be related to some humoral factors.

Indeglia and associates<sup>11</sup> have in fact pointed out that the alterations in osmotic fragility following mechanical trauma are not only limited to the traumatized cells, but also exist in the entire population of untraumatized circulating erythrocytes. With regard to the radioactive chromium method, the survival curve appears to consist of two components: an initial steep slope being followed by

a much less rapid falling slope. The former corresponds to early damage of red blood cells, the latter to sublethal damage of these cells. This maximal rate of erythrocyte destruction during and just after pumping is confirmed by a recent publication of Wallace and associates<sup>18</sup> who measured coproduction to quantify mechanically induced extravascular hemolysis.

Counterpulsation implies several requisites for the production of disseminated intravascular clotting. The blood is repeatedly circulated over foreign surfaces for a period of time which may induce activation of the Hageman and other surface clotting factors. Red cell thromboplastin liberated by hemolysis can also promote coagulations especially of stagnant acidotic blood; disintegration of platelet leads to availability of platelet factor III which also stimulates the production of clotting. Decrease of platelets and fibrinogen during counterpulsation is probably due to consumption of these factors in a coagulation process.

The resulting intravascular clotting could stimulate the release of excessive fibrinolytic activity leading to elevation of plasmin activity. Similar investigations were carried out by Gans and co-workers<sup>9,10</sup> in extracorporeal circulation. They ascertained that heparin in normal dose is probably of little value for preventing disseminated intravascular clotting, and that excess heparin is probably necessary to avoid a hypercoagulability of pumped blood exposed to certain foreign surfaces. It is well known that pathologic changes such as the demonstration of intravascular thrombi are not necessary to conclude an intravascular clotting syndrome. Clotting changes are typical and sufficient in order to diagnose this syndrome. Release of plasminogen activators and heparinization are probably two limiting factors of the intravascular coagulation syndrome in our experiments.

## CONCLUSIONS

Synchronous cardiac assistance by counterpulsation is a mechanical device to assist the failing heart for relatively short periods. In this paper, physiological modifications of the blood after such pumping are studied. Red cell destruction does not exceed the tolerance to free hemoglobin nor the ability to regenerate red cells. Moderate clotting deficits of disseminated intravascular coagulation without pathological evidence is a constant feature. We think that these hematological modifications are but of slight consequence when carrying out counterpulsation.

## SUMMARY

Haematological modifications during counterpulsation (Simas pump) are studied in the dog. An improved technique for collection of blood samples in the dog is first described. Three haematological syndromes are studied: 1) erythrocyte trauma, 2) intravascular clotting and 3) fibrinolysis. The more striking features are a diminution of apparent half life of the erythrocytes ( $Cr^{51}$ ) reaching one third to one half; biologic signs of intravascular clotting without thrombi in the capillaries and a low activation of the fibrinolytic system.

## RÉSUMÉ

Les auteurs ont étudié les modifications hématologiques consécutives à des séances de 4 heures de contre-pulsation. Cette expérimentation a été réalisée sur le chien avec une pompe SIMAS.

Une technique sûre a d'abord été mise au point pour le prélèvement à long terme des échantillons sanguins.

Trois syndromes hématologiques ont été étudiés: l'altération des hématies, la coagulation intravasculaire et la fibrinolyse. Le taux d'hémoglobine libre et la résistance osmotique globulaire varient

peu. La modification principale consiste en une diminution de 30 à 50% de la demi-vie apparente des hématies marquées au  $\text{Cr}^{51}$ .

On relève également des signes biologiques de coagulation intravasculaire sans mise en évidence de thrombi intracapillaires, ainsi qu'une légère activation du système fibrinolytique.

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