EXPERIMENTAL STUDIES ON BLEEDING DIATHESIS NOT UNCOMMONLY ACCOMPANIED WITH EXTRACORPOREAL CIRCUIATION

by

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I. INTRODUCTION

Since BIGELOW[6] and SWAN[57] claimed of hypothermic anesthesia as a available medium for the prolongation of circulatory arrest in 1950, its clinical applications have been reported by many authors. HIKASA[16)~21)] et[55) 56)] al., in our laboratory, have demonstrated that providing the patient with special treatment prior to this promising method was indispensable for successful management of the operative procedures and for the avoidance of lung complication.

On the other hand, GIBBON made out, for the first time, so called artificial heart-lung machine being substitutive for actual function of heart and lung in 1937, and from that time, laborious experimental studies had been performed towards its practical use. Finally in 1957, GIBBON succeeded in operative correction of atrial septal defect under cardiac bypass by means of the extracorporeal circulation, and thereafter, a way to its clinical application has been established by LILLEHEI[33] and KIRKLIN.[30]

Postoperative bleeding diathesis, one of the most unpleasant complications during or after the extracorporeal circulation, is still remained to be resolved thoroughly. KAULLA[67] related that a marked increase of fibrinolytic activity and appearance of a circulating anticoagulant could considerably deviate the clotting mechanism during open heart surgery with pump oxygenator. And PERKINS[49] concluded that postoperative bleeding syndrome could be produced by at least three different mechanism : (i) insufficient or excessive protamine (ii) thrombocytopenia (iii) fibrinolysis related to the surgical procedure itself. The author and colaborators thus engaged in the experimental studies of extracorporeal circulation employing a WAUD-SALISBURY's foam oxygenator and a original pulsatile pump which was newly constructed in our laboratory.

But our initial experiments ended in such disappointing results that all the experimental animals were lost apparently because of hemothorax for which, we thought, bleeding diathesis might be responsible. Therefore, the author investigated the changes in the clotting mechanism during and after extracorporeal circulation.

The following determinations on the clotting mechanism were performed in the dogs, who underwent cardiopulmonary bypass procedures with or without hypothermia : (1) Prothrombin consumption test, (2) Prothrombin time, (3) Fibrinogen value, (4) Thrombelastography, (5) Fibrinolysis, (6) Hemolysis, (7) Platelet count.

In the course of the investigation on the clotting disturbances, several improvements in our heart-lung machine have been made and finally we were able to perform the bypass procedure successfully. In this paper the author presented the analysis of bleeding diathesis which was encountered in our bypass procedure, and also the process of the improvements in our apparatus was reported.

II. MATERIAL AND METHODS

As experimental animals, adult mongrel dogs weighing 7 kg to 13 kg were used. In the initial experiments, donors were anesthetized with intravenous Pentobarbital Sodium, and the priming blood was drawn from the femoral artery after heparinization in a dosage of 2 mg/kg of bodyweight. However, in the later experiments, the blood was drawn from the donors without anesthesia in order to avoid the introduction of the anesthetic agent into the recipient. Each 500 cc of blood was collected in the silicone-coated bottle containing 20 mg of heparin. However, silicone coating had not been done in the initial experiments. Silicone coating was achieved by immersing the material into a 4% Toluene solution of DOW-CORNING 1107 silicone [27] Silicone rubber tubes were provided from NIPPON KETSUEKI Kenkyusho. As an arterial pump, a pulsatile pump,[25] [43] [45] [58] which was newly constructed by Dr. G. KAMIMOTO, Professor of the Faculty of Technology, Kyoto University, was used, and a Sigma motor pump was used in the venous circuit. At the latter half of these experiments, vertical double layered oxygenators manufactured in NIPPON KETSUEKI KENKYUSHO, were used instead of a WAUD-SALISBURY's oxygenator.

The experimental animal that could be freed from the suspicion of filaria parasitism was anesthetized with intravenous Pentobarbital Sodium (25～30 mg/kg of body weight). After the tracheal intubation, anesthesia was maintained with ether oxygen mixture in the closed system. 3 mg of heparin per kg of body weight was given intravenously prior to cannulation. The flow rate of the extracorporeal circulation varied from 40 cc to 55 cc/kg of body weight in the total perfusion, and from 15cc to 35 cc/kg of body weight in the partial perfusion. After the completion of the extracorporeal circulation, protamine sulfate was given intravenously to neutralize extrinsic heparin.

III. EXPERIMENTAL PROCESS

In the initial series of our experiments in which our pulsatile pump and a WAUD-SALISBURY's foam oxygenator were used, all the experimental animals died within a few hours after perfusion whether a cardiac bypass was partial or total. In these cases, endless bleeding was always seen in the chest cavity and other operative wounds. And hemorrhage and petechiae were observed in the nasal or tracheal mucous membrane following a minimum trauma or pressure. In autopsy, petechial bleedings were found in the mesenterium. Since the administration of protamine sulfate and all the hemostatic preparations was considered to be ineffective, we thought that surgery might be responsible for the excessive bleeding.

Therefore, we performed the experiments under partial perfusion by mean of V-A shunt without accompanying the thoracotomy (Fig. 1A and 1B). Though partial perfusion

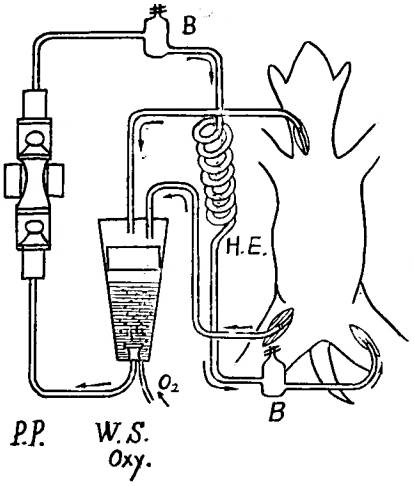

Fig.1. A Extracorporeal circuit without thoracotomy.
P.P. : Pulsatile Pump
W.S.Oxy. : Waud-Salisbury's Oxygenator
H. E. Heat-Exchanger
B. Bubble trap

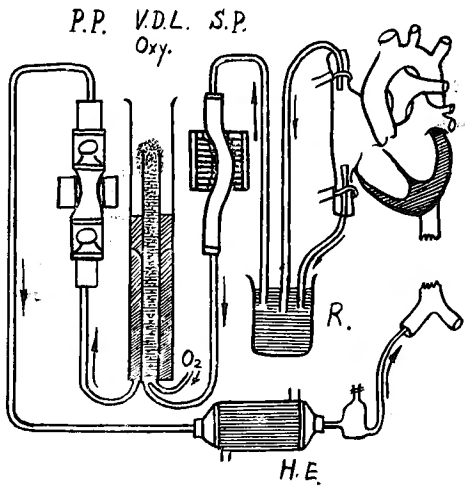

Fig.1. B Extracorporeal circuit in our latter experiments.
P. P. Pulsatile Pump
V.D.L.OXy. Vertical Double Layered Oxygenator
S. P. Sigma Motor Pump
R. Reservoir
H. E. Heat Exchanger

was performed for only 10 minutes, all the experimental animals died within a few hours after the procedure. In these cases, there was also an uncontrollable bleeding from the incised wounds previously made for arterial and venous cannulation even after the neutralization of intracorporeal heparin.

Accordingly, it was thought that the pump, the oxygenator, the connecting-circuit and the improper blood taking method might be singly or in combination causative of such impairment of the blood clotting mechanism. As the first step, the pump was taken up. A Sigma motor pump and a pulsatile pump were examined. Ten minutes partial perfusions were tried with these two types of pumps. In these experiments, all the experimental animals of both groups were lost. Since a Sigma pump is generally used successfully in many other medical centers, other factors, rather than the pump itself, were thought to be responsible for dog's death. Therefore, we examined the following factors :

1) As the priming blood was drawn into the uncoated containers, the damage of the blood might have already occurred prior to perfusion or the fragility of the blood cells might have already increased to a extent that they were easily hurt with the perfusion procedure only.

2) Two bubble traps in the circuit were not coated.

3) Polyvinyl tubes used as connecting circuit were inadequate.

With the aim of minimizing the blood trauma, the blood was taken into the silicone-coated containers. Consequently, in the experiments of 10 minutes partial perfusion, the long term survivals of animals were made possible without revealing any significant bleeding diathesis. At the next step, total cardiopulmonary bypass was done with a WAUD-

SALISBURY's foam oxygenator. In spite of only 10 minutes perfusion, a recipient succumbed to the severity of intrathoracic bleeding. Since a stainless steel ball, taking a part of valve structure in a pulsatile pump, which had not been coated, might be responsible for the unsuccessful result, silicone-coated one was used in the later experiments.

KAULLA and SWAN[67) 68)] emphasized that severe bleeding tendency might be caused during or after a cardiac bypass as a result of the enhanced fibrinolytic activity. Considering this fact, Ipsilon (antiplasmin agent) was added to the priming blood with P.V.P. which is a kind of plasma expanders (20cc of 5% Ipsilon and 30cc of 6% P.V.P. were added to 500cc of the priming blood). Then, the problems of (1) and (2) were examined by following fundamental experiments without perfusing the living body.

(i) The blood drawn into the silicone-coated bottles containing Ipsilon and P.V.P.

(ii) The blood drawn into the uncoated bottles, as the control.

The priming blood was divided into above mentioned two groups. At first, the former blood was circulated through the pulsatile pump circuit only without perfusing the body for 2 hours and the latter blood was circulated through the Sigma pump circuit similarly (as illustrated in Figs. 2A and 2B, the connecting-circuits were polyvinyl tubes, and two bubble traps were not coated at this time). The degree of hemolysis and the fibrinogen content were measured prior to and after circulation. After circulation, the degree of hemolysis of the former and latter blood were 159mg%, 505mg% respectively and the fibrinogen content were 155mg%, 82mg% respectively. Secondly, the former blood was circulated through the Sigma pump circuit from which a bubble trap was removed, and the untreated latter blood was circulated through the same circuit by the pulsatile pump (connecting-circuit was polyvinyl tube and a bubble trap was coated since this time). As a result, the hemolysis was less in the blood of the first group, however the reduction of the fibrinogen content was minimum in both groups; the degree of hemolysis in two groups were 127mg%, 177mg% and fibrinogen content were 492mg%, 399mg% respectively (Figs. 3A and 3B).

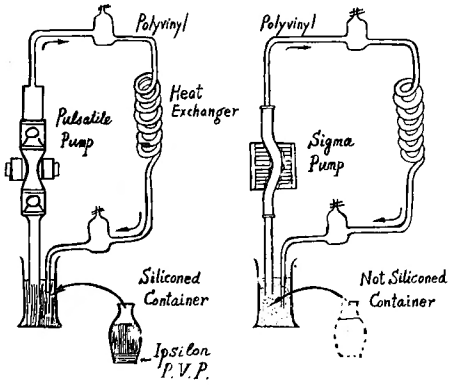

Fig. 2 A

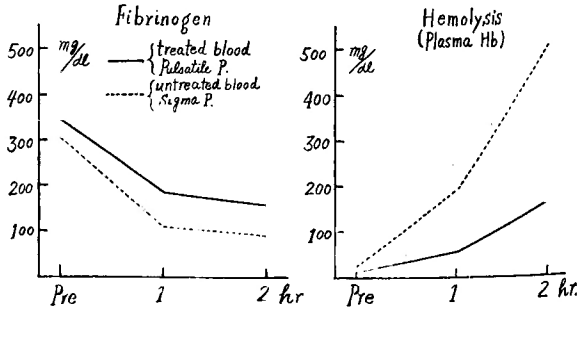

Fig.2 B

Fig. 2 A The treated blood was circulated through the pulsatile pump circuit only without perfusing the body for 2 hours and the untreated blood was circulated through the Sigma pump circuit similarly. The connecting-circuit were polyvinyl tubes, and two bubble traps were not coated at this time.

Since the treated blood showed the excellent result as the priming blood, only this type of blood was used in the subsequent circulation through the Sigma pump circuit as well as through the pulsatile pump circuit. The result showed that there was the minimum hemolysis in both groups with the plasma hemoglobin concentration of about 90mg% (Figs. 4A and 4B).

Finally, the problem of (3) was investigated. Polyvinyl tubes used as connecting-circuits were substituted by silicone rubber tubes and the previously described two sorts of blood were circulated through this new pulsatile pump circuit for 2 hours. Though the reduction of fibrinogen was much the same as previous trial, the dramatic improvement in hemolysis was seen to the extent of 17mg% in the treated blood and of 26mg% even in the untreated blood (Figs. 5A and 5B). Based on the results of these fundamental experiments, a total perfusion in the animal was carried out with the treated blood described previously. Fortunately, bleeding tendency during the procedure was markedly

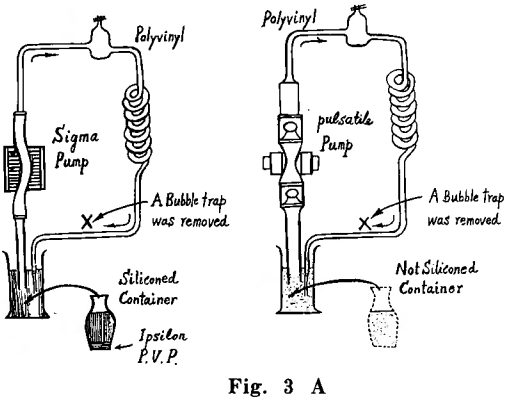

Fig. 3 A

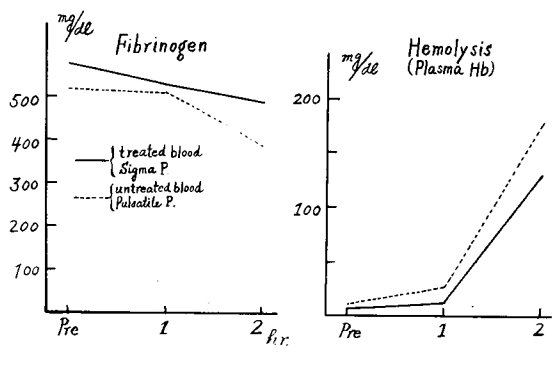

Fig. 3 B

Fig. 3 A The treated blood was circulated through the Sigma pump circuit from which a bubble trap was removed, and the untreated blood was circulated through the same circuit by the pulsatile pump. The connecting-circuit was polyvinyl tube, and a bubble trap was coated since this time.

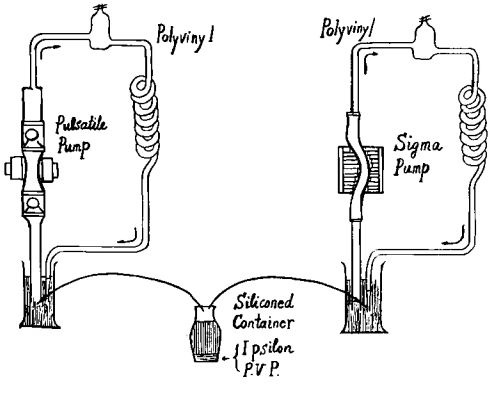

Fig. 4 A

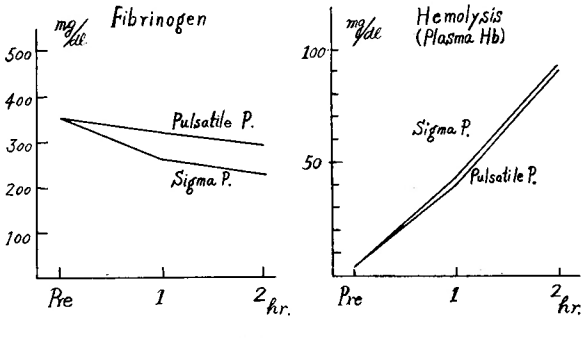

Fig. 4 B

Fig. 4 A The treated blood was circulated through the Sigma pump circuit as well sa the pulsatile pump circuit.

diminished and we could get a first survivor after 10 minutes total perfusion. We attempted to accumulate the long term survival cases even after the more prolonged cardiac bypasses under the normothermic condition. But all the animals died after the prolonged extracorporeal circulation with our WAUD-SALISBURY's foam oxygenator, though the hemolysis was minimum (Table 1.). Then, our thoughts were led to a trial of the rapid hypothermic method employing no oxygenator.[13) 51) 62)] The rapid hypothermic

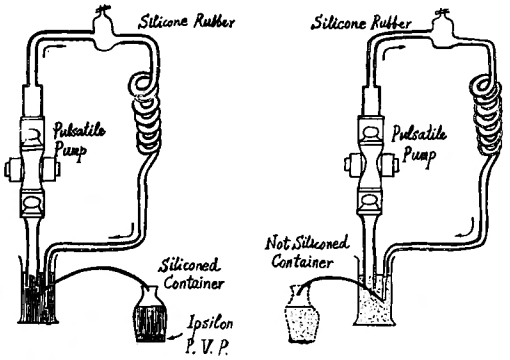

Fig. 5 A

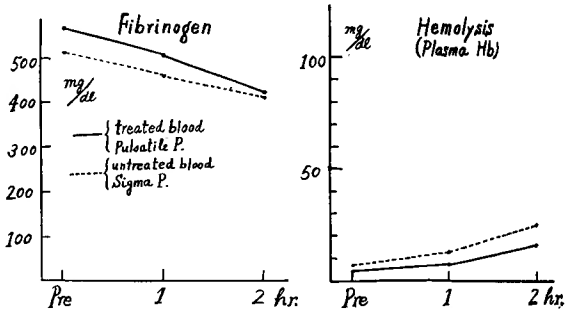

Fig. 5 B

Fig. 5 A Polyvinyl tubes used as connecting-circuit were substituted by silicone rubber tubes and two sorts of blood were circulated through this new pulsatile pump circuit. The dramatic improvement in hemolysis was seen.

Table 1 GROUP (1)

(i) Extracorporeal Circulation Employing No Oxygenator

Dog No.	Weight & Sex	Fate	Perfusion Time	A.P.	Flow Rate (/m.kg.)	Bleeding Tendency	Cause of Death	Improvement
2	12.6kg M.	Death 15 hr.s	10 min.	P. P.	15cc	**	Bleeding Diathesis	
3	7.2kg M.	Death 12 hr.s	10 min.	S. P.	20cc	***	Bleeding D. Filaria	
4	6.7kg F.	Death 5 hr.s	10 min.	P. P.	25cc	***	Bleeding Diatheais	
5	8.5kg. F.	Survived	10 min.	S. P.	35cc	.....		(1) Coating of Container & Circuit
7	10.3kg M.	Survived	10 min.	P. P.	30cc	.....		(1)

(ii) Extracorporeal Circulation Employing Waud-Salisbury's Oxygenator

8	5.9kg M.	Death 25 hr.s	10 min.	S. P.	35cc	.....	Unknown	(1)
9	10.0kg M.	Survived	10 min.	P. P.	35cc	.....		(1) (2) Addition of Ipsilon & P. V. P
10	7.3kg M.	Survived	10 min.	P. P.	25cc	.....		(1) (2)
11	6.6kg M.	Death 26 hr.s	10 min.	P. P.	45cc	.....	Microthrombus	(1) (2)
12	7.5kg M.	Survived	10 min.	P. P.	50cc	.....		(1) (2)

A. P. : Arterial Pump P. P. : Pulsatile Pump S. P. : Sigma Motor Pump
M. : Male F. : Female

Table 2 GROUP (2) Rapid Hypothermic Method (A-A Shunt)

Dog No.	Weight & Sex	Fate	Esoph. Temp. (°C)	Rectal Temp. (°C)	Cooling Time (min.)	Rewarm. Time (min.)	Perfusion Time (min.)	Cause of Death	Side of Cannulation
13	6.6kg M.	Death 2 hr.s	18.5	24.5	33	87	120	Heart failure	Left
14	12.9kg M.	Death 3 hr.s	23.0	25.7	51	75	126	Unknown	Left
15	7.8kg M.	Death 5 hr.s	20.4	21.0	58	80	138	Unknown	Left
16	10.0kg M.	Death 5 hr.s	22.9	24.3	45	53	98	Unknown	Left
17	5.5kg M.	Death 7 hr.s	15.8	20.0	32	61	93	Unknown	Left
18	6.0kg F.	Survived	22.0	19.8	46	87	133		Right
19	9.0kg M.	Survived	22.0	24.5	24	41	65		Right

method was tried in 7 animals by mean of A-A shunt. The blood was drained from the femoral artery and pumped to the systemic circulation via the carotid artery after passing through the heat-exchanger. The esophageal temperature of the dog was lowered to about 20°C and rewarmed after 10 minutes cessation of the perfusion. Only 2 animals which received the blood supply via right carotid artery could survive, and other 5 animals delivered the blood via the carotid artery of the left side died within 7 hours after the perfusion.

The reason of different results in both the experiments was explained in TATSUDA's paper[59]. Of course, no remarkable bleeding diathesis was noticed during or after those hypothermic methods (Table 2).

Accordingly, it was decided to adopt a disposable double layered oxygenator instead of a WAUD-SALISBURY's foam oxygenator. The inner surface of this oxygenator was coated before each trial. When the total perfusion was performed under the combination of the pulsatile pump and this new oxygenator, the experimental animals resuscitated from anesthetized state more promptly and survived. Moreover, intrathoracic bleeding was entirely conquerred (Table 3).

IV. EXPERIMENTAL RESULTS

The clotting mechanism was studied by the determinations of the following factors :

1) Prothrombin consumption test,
2) Prothrombin time (modified QUICK's method),
3) Fibrinogen value (tyrosin method),
4) Thrombelastography,
5) Fibrinolysis (fibrin plate method and thrombelastography),
6) Hemolysis,
7) Platelet count.

1) Prothrombin consumption test ;

To inquire abnormality in the first phase of coagulation, prothrombin consumption test was adopted. This test was carried out in 7 normothermic dogs that underwent total

Table 3 GROUP (3)

Dog No.	Weight & Sex	Fate	Oxygenator	Perfusion Time (total)	Flow rate cc/m.kg.	Cause of Death	Improvement
21	5.5kg M.	Death 2 hours	W.-S. Oxy.	10 min.		Hemothorax Hyperthermia	
22	7.5kg M.	Death 5 hours	W.-S. Oxy.	10 min.		Atelectasis, Intraabdominal Bleeding	
23	6.3kg M.	Death 5 hours	W.-S. Oxy.	10 min.		Hemothorax Filaria	
24	8.0kg F.	Death justa fter	W.-S. Oxy.	10 min.		Technical failure	
25	11.2kg M.	Survived	V.D.L. Oxy.	15 min.	40cc/m. kg.		(1) New Oxy. (2) E. C.
26	7.2kg M.	Death 5 hours	V.D.L. Oxy.	15 min.	40cc/m. kg.	Unknown	(1) (2)
27	6.7kg F.	Death 2 hours	V.D.L. Oxy.	15 min.	45cc/m. kg.	Hypovolemia	(1) (2)
28	9.7kg F.	Survived	V.D.L. Oxy.	15 min.	45cc/m. kg.		(1) (2)
29	7.5kg F.	Death 12 hours	V.D.L. Oxy.	15 min.	40cc/m. kg.	Filaria	(1) (2)
30	8.5kg F.	Survived	V.D.L. Oxy.	18 min.	45cc/m. kg.		(1) (2)
31	10.0kg M.	Survived	V.D.L. Oxy.	15 min.	50cc/m. kg.		(1) (2) (3) Coating of Oxygenator
32	9.0kg F.	Survived	V.D.L. Oxy.	30 min.	55cc/m. kg.		(1) (2) (3)
33	9.9kg M.	Survived	V.D.L. Oxy.	30 min.	50cc/m. kg.		(1) (2) (3)
34	7.5kg M.	Death 3 hours	V.D.L. Oxy.	30 min.	50cc/m. kg.	Technical failure	(1) (2) (3)
35	9.6kg F.	Survived	V.D.L. Oxy.	30 min.	50cc/m. kg.		(1) (2) (3) (4) Addition of A.C.D.Solution.
36	9.9kg F.	Survived	V.D.L. Oxy.	25 min.	40cc/m. kg.		(1) (2) (3)

W.-S.Oxy. : Waud-Salisbury's Oxygenator
V.D.L.Oxy.: Vertical Double Layered Oxygenator
E. C. : Electro-Cautery
A. C. D. : Acid Citrate Dextrose

Table 4 Thromboplastin Before and After E. C. C. (Normothermia)

Dog No.	Prothrombin Time Before Perfusion (sec.) Plasma	Serum	Prothrombin Consumption	Judge	Prothrombin Time After Perfusion (sec.) Plasma	Serum	Prothrombin Consumption	Judge
22	15.2	(-)	Sufficient	Normal	17.5	(-)	Sufficient	Normal
23	21.3	(-)	Sufficient	Normal	17.5	105.2	Sufficient	Normal
24	12.2	(-)	Sufficient	Normal	14.4	22.4	60%	Reduced
25	18.6	(-)	Sufficient	Normal	21.8	65.2	Sufficient	Normal
26	15.2	(-)	Sufficient	Normal	12.8	92.6	Sufficient	Normal
29	13.4	(-)	Sufficient	Normal	17.5	27.2	44%	Reduced
32	12.4	(-)	Sufficient	Normal	17.6	23,1	34%	Reduced

The distinct reduction of thromboplastin in the post-pump period was found in 3 dogs.

cardiac bypass for the period of 10～30 minutes. High prothrombin value of serum suggests the diminution of thromboplastin. The distinct reduction of thromboplastin in the post-pump period was found in 3 dogs (Table 4).

2) Prothrombin time;

In the normothermic group, prothrombin time was determined about the specimens taken from the priming blood and from the recipient before and after the perfusion. In the rapid hypothermic group, the measurements were performed at three occasions, i. e; before pump, after cooling and after rewarming. In normothermic group, prothrombin times, except a few cases, showed tendency to become prolonged at the post-pump period, but never exceeded those of the priming blood. In the rapid hypothermic group (18～23°C), prothrombin time at the post-pump period was distinctly shortened as compared with those of the normothermic group. It is known that prothrombin time is also prolonged in the deficiency of factor V, VII and by the presence of antithrombin (Fig. 6).

3) Fibrinogen value;

Fibrinogen contents at pre- and post-pump period were measured by tyrosin method using the electrophotometer. In the initial experiments in which due attention was not paid for fibrin deposition in the apparatus, the fibrinogen content at post-pump period decreased to the extent of 100mg%, which was evidently responsible for the impairment of coagulation. After the improvement of the apparatus was made, fibrinogen loss was kept in the range of 20%～30% of the preperfusion level in the normothermic group with the perfusion of about 30 minutes However, the perfusion time of 1～2 hours was necessary in the hypothermic group because our initial heat-exchanger was not so effective as BRAWN-HARISON[8] type which was employed later. As a result, fibrinogen loss ranged from 80 to 100mg% (Fig. 7).

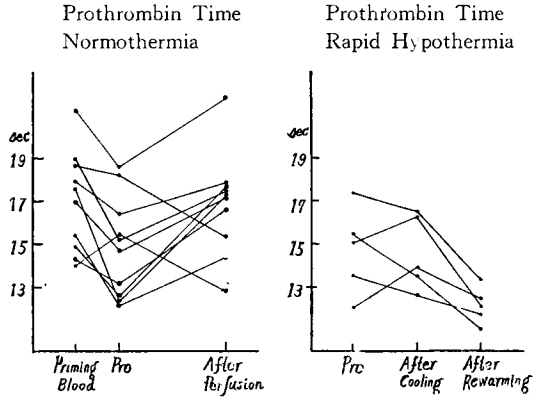

Fig. 6 In normothermic group, prothrombin times, except a few cases, showed tendency to become prolonged at the post-pump period, but never exceeded those of the priming blood. In the rapid hypothermic group (18～23°C), prothrombin time at the post-pump period was distinctly shortened as compared with those of the normothermic group.

4) Thrombelastography;[10)66)]

HARTERT[15] invented a thrombelastography by which recording of the changes in physical characteristics of the clot during its development and also its dissolution was made possible. We used this apparatus and the usual three components of the thrombelastogram were evaluated, i. e; the reaction time r, the clot formation time k, and the maximal amplitude ma. In the normothermic group, the values of r and r+k had a general trend of moderate shortening but the values of ma were slightly reduced. Consequently it was interpretted that the blood coming into direct contact with air and oxygen, passing through the bubble oxygenator, had an inclination to clot, though the firmness of

clot was diminished after the perfusion. In the hypothermic group, the values of both r and r+k were prolonged in proportion to the lowering of the blood temperature, whereas the values of ma were reduced in contrast to those of r and r+k, and yet after the rewarming period, the values of r and r+k were returned to the almost preperfusion level. It was interpretted that the development of clot was slow under hypothermia, but this tendency was reversed by rewarming (Figs. 8, 9 and 10).

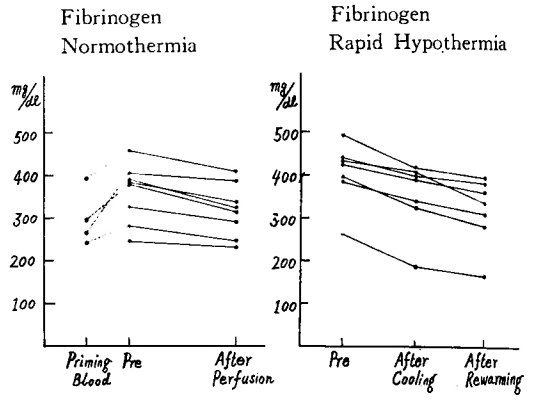

Fig. 7 Fibrinogen loss was kept in the range of 20%～30% of the preperfusion level in the normothermic group with the perfusion of about 30 minutes. In the hypothermic group, fibrinogen loss ranged from 80 to 100mg%.

5) Fibrinolysis;

The homogenous fibrin plates were prepared by pouring 0.1% fibrinogen solution into PETRI's dishes. A drop of sample, sometimes serum itself and sometimes euglobulin fraction precipitated at pH 5.2, was placed on these plates. Fibrinolytic activity was estimated from the lysis evoked area after 18 hours[1)5)63)]. Some of these plates were heated in 85°C for 30 minutes to eliminate the effect of activator. In most of the cases in which Ipsilon was added to the priming blood, no marked increase of fibrinolytic activity was seen at post-pump period. But in control cases without Ipsilon in the priming blood, considerable increase of fibrinolytic activity was recognized at post-pump period. However, the inhibitory action of Ipsilon for plasmin system was not appreciated after 20 hours from its use.

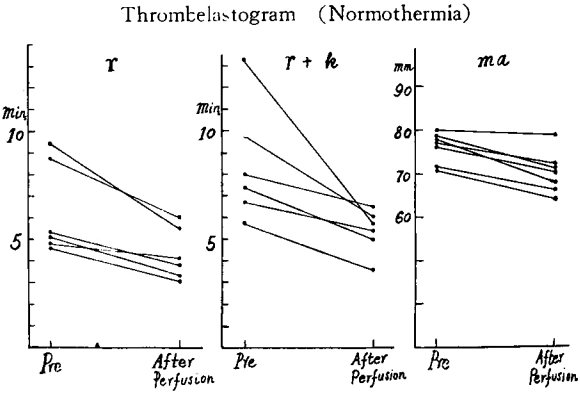

Fig. 8 In the normothermic group, the values of r and r+k had a general trend of moderate shortening but the values of ma were slightly reduced.

Thrombelastography also provides with a useful information in regard to fibrinolytic activity. No spontaneously induced fibrinolysis was found in our experiments. Streptokinase prepared from Streptokinase-Streptodornase, LEDERLE, and human plasma were utilized for the artificial activation of the plasmin system.[41)42)61)69)] 50～100 units of S. K. per cc of the blood, by the aid of 0.04cc of human plasma, was potent enough to demonstrate a characteristic pattern of fibrinolysis in coagulogram at pre-pump period. However, at post-pump period, such typical fibrinolysis in coagulogram was not seen in the cases in which Ipsilon was used. And in the control cases lacking Ipsilon, fibrinolytic tendency at post-pump period was intensified upon the addition of same dose of S. K. and human

plasma (Figs. 11, 12 and 13).

6) Hemolysis;

The degree of hemolysis (plasma hemoglobin concentration) was measured by the electrophotometer. After the improvements of the extracorporeal circuit, the degree of hemolysis after the perfusion was more or less 15mg % in most of the cases, and it never increased beyond the level of 30mg% in both groups of normothermic and hypothermic method.

7) Platelet count;

The platelet count was determined by the direct method using REE & ECKER's solution. The data will be described in chapter V.

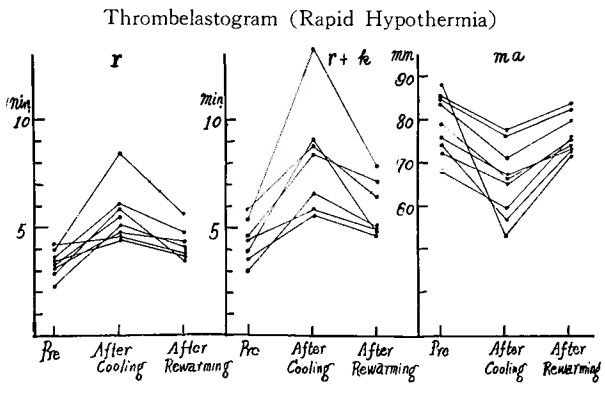

Fig. 9 In the hypothermic group, the values of both r and r+k were prolonged in proportion to the lowering of the blood temperature, whereas the values of ma were reduced in contrast to those of r and r+k, and yet after the rewarming period, the values of r and r+k were returned to the almost preperfusion level

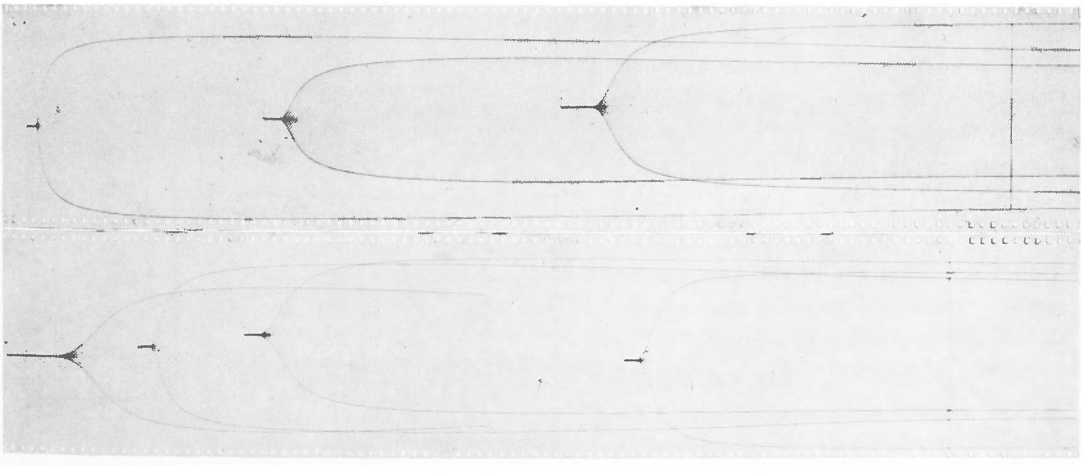

Fig. 10. Influence of rapid hypothermia on the coagulogram.
Upper: Priming blood, After cooling, After rewarming. (from left to right)
Lower · Before E. C. C., Priming blood, After cooling, After rewarming. (from left to right)

V. DISCUSSION

In the early series of our experiments in which our pulsatile pump and WAUD-SALISBURY's foam oxygenator were used, all of our experimental animals were lost within a few hours after the perfusion from hemothorax following bleeding diathesis. We assumed that this disturbance of blood clotting might have been resulted from inadequacy in the pump-oxygenator, incorrect blood taking method and improper connecting-circuit which might have caused mechanical and chemical trauma to the blood. Therefore we performed a series of experiments, as previously described, to elucidate the problem.

Generally speaking, the more complicated function a organ has, the subtler mechanism

of adjustment is developed in it. Blood is also assumed to be a complicated organ which plays an important role for the maintenance of homeostasis. Blood has apparently incompatible two aspects; the maintenance of liquidity on one hand and the clotting on the other. It may be imagined that the homeostasis of the blood is more easily disturbed when the blood is circulated in the extracorporeal circuit than in the vessels. Although the coagulation theory of MORAWITZ was a classical one, there has been a remarkable progression in our knowledge in blood clotting mechanism since the discovery of Vitamin K in 1934. In the contemporary theory of blood clotting, numerous factors have been mentioned[7)35)]. In view of this contemporary coagulation theory, our cardiac bypass system was examined and improvement has been made.

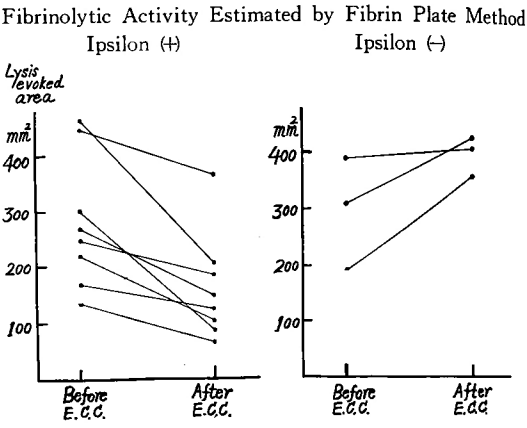

Fig. 11 In most of the cases in which Ipsilon was added to the priming blood, no marked increase of fibrinolytic activity was seen at post-pump period. But in control case without Ipsilon in the priming blood, considerable increase of fibrinolytic activity was recognized at post-pump period.

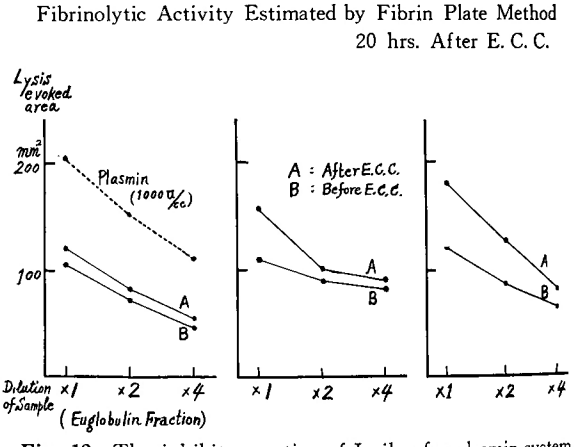

Fig. 12 The inhibitory action of Ipsilon for plasmin system was not appreciated after 20 hours from its use.

First of all, the quality of the materials coming into direct contact with the circulating blood was examined. The clean, smooth and nonwettable inner surface of the circuit is required. PERKINS[48)], using a originally devised method, determined the clotting times of whole fresh blood exposed to various materials that might be employed as pump-oxygenator circuit. He reported the superiority of inert nonwettable surfaces in delaying the coagulation of blood as compared with untreated glass surface and that the uncoated glass surface played an active role in speeding the coagulation. And further described that there was a moderate prolongation of the clotting time of whole fresh blood in polyethylene as compared with polyvinyl, in Parafilm as compared with Pliofilm, and in arquad-treated steel as compared with the untreated metal, and that no difference was found between silicone-coated glass and polystyrene, between polyvinyl and silicone rubber, nor between Parafilm and silicone rubber. However, according to the results of our experiments, dramatic reduction of hemolysis after the circulation was seen in the cases in which the connecting tube of silicone rubber instead of polyvinyl tube was used. So it can be said that trauma to the

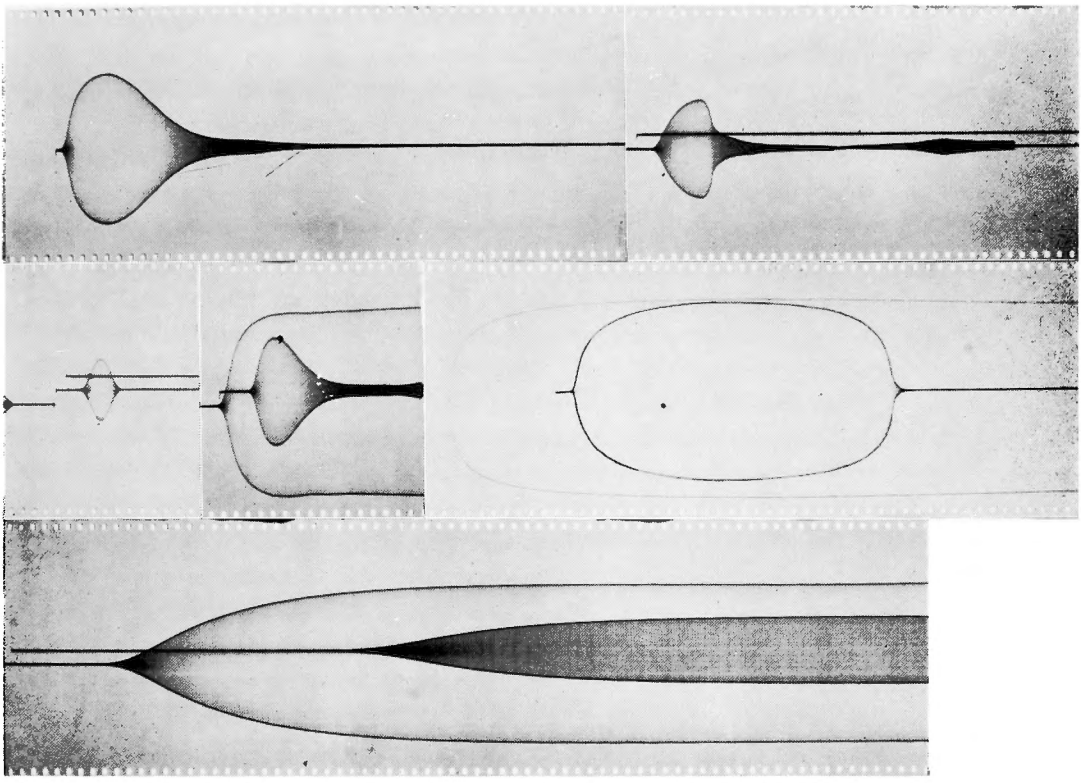

Fig. 13. Influence of Streptokinase on the coagulogram before and after extracorporeal circulation.
Upper : Left ; With Streptokinase activated fibrinolysis before E. C. C. (50～100 U. of S. K. and 0.04ml. of human plasma were added to Iml. of whole blood)
Right ; Intensified fibrinolysis after E. C. C. in which Ipsilon was not added to the priming blood. (same dose of S. K. and human plasma were added)
Middle : Various dgrees of fibrinolysis were seen before E. C. C. upon the addition of S. K. and human plasma. (same dose)
Lower : After E. C. C. in which Ipsil on was added to the priming blood. No characteristic pattern of fibrinolysis was obtained upon the addition of same dose of S. K. and human plasma.

blood may be less in the silicone rubber tube than in the polyvinyl tube in the dynamic state when the blood is circulated. As the mechanical turbulence of blood in the bubble traps was thought to decrease the platelet count and accelerate coagulation, a number of bubble traps was reduced to only one which was inserted prior to the arterial cannula and was newly coated in every use. Considering that the stainless steel ball valve of the pulsatile pump, designed to prevent regurgitation, acted as a protrusive foreign body within the blood stream, this ball was wrapped in silicone rubber. Therefore, all the inner surfaces of the circuit coming into direct contact with the circulating blood were silicone-coated.

The first phase of coagulation is initiated by the breakdown of platelets. Thromboplastinogenase being platelet III factor flows out when the continuity of blood vessels is lost and reacts with the antihemophilic globulin (thromboplastinogen) contained in the globulin

fraction of the serum protein and is finally converted into the thromboplastin in the presence of plasma thromboplastin complex and Ca ions. Abnormalities in the first phase of clotting mechanism are reflected upon the prothrombin consumption. A. PERKINS[49] described that his scattered test of the prothrombin time and prothrombin consumption on the patients who had received the minimum necessary dose of protamine have revealed results in a completely safe range. But in our experiments under normothermic condition, in 3 out of 7 animals, a depletion of the thromboplastin of from slight to considerable degree was found at the post-pump period only and no abnormality at the pre-pump period was detected. Since the platelet count is most sensitive to trauma and artificial surfaces and since the serum protein usually deviates in far less degree than the former[40)60)], it might be reasonably said that the depletion of the thromboplastin after the perfusion was due to the diminution of platelet III factor. KIRBY[28] found that the platelet count in the priming blood during pooling might fall to 100,000 or even 60,000. WESOLOWSKI[71], GOLLAN[12] and ALLEN[4] described a marked reduction in the platelet count after perfusion sometimes to the extent of 50% of preperfusion value. In facts, the platelet count in the priming blood in the silicone-coated containers diminished to 70,000～60,000 after 3 hours pooling, and its count in the body after perfusion was reduced to more or less 100,000～70,000 in authors's scattered tests. However, at the present time, it might be impossible to avoid the reduction of the platelet count. Moreover, PERKINS[49] reported that no marked difference in platelet count reduction was found between bubble and film oxygenator, and there had been no improvement in the problem of platelet loss in the past 2 years When a reduction of the thromboplastin is suspected, the administration of thromboplastin preparation is desirable HIBINO[22] and NYGAAD[44] mentioned that bleeding tendency was not apparently disclosed unless the platelet count dropped below 30,000, but thrombocytopenia was also responsible for the impairment of normal function of capillaries, which might contribute to the appearance of a hemorrhagic symptom[39]. The presence of pyrogen in the blood is executive of the depression of the platelet count as well as the fibrinogen content and executive too of the enhancement of fibrinolytic activity, so in the extracorporeal circulation of experimental animal, aseptic procedure must be born in mind in order to avoid bleeding tendency[70]. Platelets have a capacity to bind the action of administered heparin, therefore, the potency of heparin increases in proportion to the reduction of platelet count. For this reason, when marked reduction of platelet count occurs, larger dose of protamine must be given to neutralize intracorporeal heparin[3].

In the second phase of coagulation, prothrombin is further converted to thrombin by the aid of thromboplastin. Marked changes in prothrombin values before and after perfusion were not found. In some of the cases of normothermic perfusion, slight prolongation of prothrombin time was recognized but in most of the cases, it remained in the physiologic range.

The decrease of fibrinogen content in the blood was one of the causative factors of bleeding diathesis in our initial experiments of extracorporeal circulation. OSBORN[49] showed that some of the extracorporeal circulation was accompanied by fibrinogen loss and resultant hemorrhage. When a WAUD-SALISBURY's foam oxygenator was used in our initial experiments, a large amount of fibrin was depositted on the wall of the oxygenator and

fibrinogen content decreased to a value of more or less 100mg%. In the latter experiments, fibrinogen loss after perfusion was reduced to a range below 100mg% as the result of previously described improvements of our pump-oxygenator system. Since it is known that hypofibrinogenemia of this extent does not cause bleeding diathesis, fibrinogen loss was not so serious as far as undesirable clotting in the circuit was avoided. Many authors have reported the enhanced fibrinolytic activity as a cause of severe bleeding tendency during or after cardiac bypasses. And recently, more attentions have been paid to bleeding diathesis due to fibrinolysis rather than fibrinogen removal in the apparatus. Fibrinolysis is occasionally caused in the various situations such as anesthesia, surgical operations (especially operations of lung, pancreas and prostate), gynecological diseases, stress, hemorrhagic shock, emotion and anoxia[1)31)34)36)37)] etc. Afibrinogenemia or hypofibrinogenemia would occur in consequence of the digestion of clottable fibrinogen. Plasminogen is the precursor of plasmin and converted to plasmin in virtue of activator. As activator of plasminogen, tissue activator, urokinase and plasma-exudate activator have been discovered until now. Activators are also the activated forms of proactivators which are known as Streptokinase, Staphylokinase, Cytofibrinokinase existing in the cells, and Serofibrinokinase existing in the blood. The exact mechanism of plasmin activation is still remained to be solved. It is said that plasmin and activators have their own inhibitors and their interactions maintain the plasmin system in a state of equilibrium, and that plasmin in the normal plasma builds up a complex combining with antiplasmin, and enhanced fibrinolysis is revealed when this complex is broken. The ratio of combining with the inhibitory agents is varied according to the environmental conditions, and it sometimes retards, sometimes urges coagulation. Since plasmin titer could be elevated by unspecific causes such as emotional upset or anxiety for operation, moderate activation of plasmin system may occur more or less in all cases. However, correlation between plasmin titer and hemorrhagic symptoms has not been established and moderate increase of plasmin titer may not necessarily cause hemorrhagic diathesis[67)]. ALLEN[4)] showed that once fibrinolysis occurred to the last degree, fibrinogen in the blood stream disappeared within a few minutes and it took over 10 hours before fibrinogen content was restored to its normal values Therefore, it would be more important to know the maximum titer of plasmin during the procedure of cardiac bypass rather than intermittent examination of fibrinolytic activity.

It is also of extreme importance to avoid clotting in the circuit by adequate heparinization and by the use of a pump-oxygenator system with smooth inner surfaces. If coagulation in the circuit takes place, microthrombi thus produced will occlude the capillary beds and restoration mechanism to dissolve them will lead to an uninvited condition where fibrinolytic activity is raised abnormally. Moreover, the elevated fibrinolytic activity due to fibrin deposition in the oxygenator would disclose C. P. P. (capillary permeability promoting) action which ordinarily induces endless bleeding as oozing

On the other hand, fibrinolysis is said to be associated with allergic conditions[31)46)64)]. Plasmin is responsible not only for pronounced bleeding tendency due to the digestion of clotting factors such as fibrinogen and thrombin, but also responsible for the appearance of allergic or other various symptoms due to thus formed toxic polypeptide as histamin

and acetylcholine. Furthermore, NYHUS[24] suggested that the breakdown of infused white blood cells may release an excessive amount of histamin and the breakdown of donors and recipient platelets by the pump-oxygenator system may also release excessive quantities of serotonin since platelets are rich in this material. Therefore, he assumed that abnormally increased amount of these substances might be a part of etiological mechanism of cyanosis, mottling of the skin, hypotension, coma and sudden death following the use of extracorporeal circulation. In the early experiments, we were puzzled by the appearance of histamin-like response of the animals, such as hypersecretion of salivary glands, diarrheic feces and oozing which were seemingly the results of increased permeability of the capillaries.

PH of the blood of low flow rate group (75cc/kg/min.) is usually lower than that of high flow rate group (190cc～200cc/kg/min.) and anoxia (hypoxia) of the tissue is accordingly more easily invited in the former group. KAULLA[67] stated that a characteristic correlation was found between the pH of the blood and fibrinolytic activity, and so, fibrinolysis could be brought about more intensively and more persistently in low flow rate group. KWAAN[32] showed that anoxia in the arterial and venous wall would stimulate the release of activator and a large amount of adrenalin, histamin and serotonin would enhance fibrinolytic activity. Then he showed that ischemia in the wall of blood vessels due to arterial and venous vasoconstriction induced the release of plasminogen activator. MOLE[38] and VIRCHOW[65] demonstrated that fibrinolysis in the corpse suddenly died from anoxia was most characteristic in the peripheral capillaries. And OKAMOTO[47] showed that anoxia intensified the hemorrhagic diathesis produced by the administration of Streptokinase, an activator of plasminogen. Enhanced fibrinolytic activity simultaneously accompanies the increased membrane-permeability of all body.

Ipsilon is an antiplasmin preparation which was found by OKAMOTO[46] and has a powerful inhibitory action to both plasmin and activator. Ipsilon is white crystal, soluble in water but not soluble in organic solvents and has a chemical structure of ε amino-n-caproic acid. Intravenous administration of 20ml of 5% Ipsilon in human is effective to lower the elevated fibrinolytic activity to normal level[1]. When Ipsilon was added to the priming blood drawn to silicone-coated container, the exudative hemorrhagic tendency was not observed during and after the bypass procedure. We believe that Ipsilon prevented the abnormal increase of capillary permeability. Since Ipsilon has a protecting action for the diminution of resistance of the blood cells[26], its administration was considered to be useful not only for the procedure of extracorporeal circulation, in which trauma to the blood cell is unavoidable, but also for other various conditions where fragility of red cells and elevated fibrinolytic activity might occur[63]. According to the author's evaluation by fibrin plate method, fibrinolytic activity of the untreated groups was elevated at post-pump period, but in the treated cases in which Ipsilon was used, definitely depressed plasmin activity was observed at post-pump period. However, Ipsilon's effect disappeared after 20 hours from its use. So, it is advisable to administer Ipsilon intermittently every 3～4 hours in order to maintain the effect[1]. And plasmin titer was indirectly estimated from the intensity of fibrinolysis activated with Streptokinase. Since dog's activator is less sensitive to S. K. (Streptokinase) than humans, plasminogen in dogs is not easily

activated by S. K. only. But marked fibrinolysis was seen when human plasma was added with S. K[69]. Addition of 50 units of S. K. and 0.04cc of human plasma to the blood specimen taken immediately after anesthesia revealed marked fibrinolysis in coagulogram. Nevertheless the blood taken immediately after perfusion, in all cases, showed no characteristic pattern of fibrinolysis[10)66)] and no complete lysis of once formed clot was seen upon the addition of same dose of S. K. and human plasma owing to the antiplasmin action of Ipsilon which had been previously added to the priming blood.

In the studies of substitutive substance for body fluid, polyvinylpyrrolidone (P. V. P.) was synthesized by W. REPPE[50] in 1940 and the preparations of various molecular weights have been manufactured. P. V. P. has a analogical function as serum protein and DIECKHOFF[11] summarized the pharmacological action of P. V. P. as follows; hemodynamic action, detoxicating action and the reinforcement of capillary wall. Pereston-Nwhich was added to the priming blood with Ipsilon is the physiologic saline solution including 6 % P. V. P. of low molecular weight of 13,000. K HUMMEL[23] found that P. V. P. of mean molecular weight of 40,000 had a hemostatic action as powerful as thrombin, despite the coagulation process was not affected by P. V. P. Detoxicating action of P. V. P. was expected towards the toxic polypeptide as well as increased amount of some humoral substances, because the former is produced by the destruction of blood cells and serum protein, and the latter is released by defence mechanism for perfusion stress. P. V. P. was also found to depress the increased permeability at histamin shock and subsequently blood pressure could be restored easily. Therefore, if hypotension or shock developed following extracorporeal circulation, the administration of P. V. P. which has the almost same osmotic pressure as serum protein would be more advisable than giving physiologic saline or RINGER's solution. Its mechanism is thought to be the fixation of P. V. P. into the protein of cell membrane[72)73)]. Therefore, hemorrhagic diathesis was almost completely eliminated in our later experiments in which Ipsilon and P. V. P. were used. Accordingly, Ipsilon and P. V. P. were thought to be valuable medicaments for the improvement of vascular dysfunction accompanied with extracorporeal circulation.

VI. CONCLUSION

Cause of the bleeding diathesis, which was induced by extracorporeal circulation were analyzed. The following results were obtained;

1) Slight abnormalities in thromboplastin formation were found in a few cases at the post-pump period.

2) Improvements of the extracorporeal circuit eliminated a marked reduction of the fibrinogen content and also severe thrombocytopenia.

3) The vascular dysfunction associated with fibrinolytic activity, severe thrombocytopenia and histamin-like response due to toxic subsatnces were assumed to play a prime role for the development of bleeding diathesis.

4) Administration of the antiplasmin preparation of Ipsilon could normalize enhanced fibrinolytic activity during the pump-oxygenator procedure. P. V. P. which was added with Ipsilon to the priming blood, was also presumed to be efficient enough for the correction of impaired vascular function. Consequently, bleeding diathesis during or after

the perfusion disappeared and all the experimental animals, excepting a case of technical failure, survived after 30 minutes total perfusion.

During the course of investigation, the following improvements in our apparatus and in our procedures were made.

1) Silicone-coating of inner surfaces of pump-oxygenator coming to direct contact with the circulating blood, and of the container for drawing the priming blood,

2) Employing the silicone rubber tube as a connecting-circuit,

3) Addition of Ipsilon and P. V. P. to the priming blood,

4) Restricting the number of bubble traps to minimum,

5) Wrapping the ball valve of a pulsatile pump in silicone rubber,

6) Supplement of thromboplastin preparation,

7) Administration of topical thrombin and the use of electro-cautery for meticulous hemostasis of the operative field.

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REFERENCES

1) Abe, T.: An Antiplasmin Preparation and Its Clinical Application. Jap. J. C. H., **1**, 433, 1960.
2) Alkjaersig, N., Fletcher, A. P., & Sherry, S.: ε-amonocaproic acid, An Inhibitor of Plasminogen Activation. J. Biol. Chem., **234**, 832, 1959.
3) Allen, J. G., Moulder, P. V., Elghammer, R. M. & Grossman, B. J.: A Protamine Titration as An Indication of a Clotting Defect in Certain Hemorrhage. J. Lab. and Clin. Med., **34**, 473, 1949.
4) Allen, G.; Problems and Questions on Coagulation of Blood Arising From the Use of Extracorporeal Circulation Pumps and Oxygenators. In Allen ed.: Extracorporeal Circulation. Springfield Ill. Charles Thomas, 1960.
5) Astrup, T. & Müllertz, S.: The Fibrin Plate Method for Estimating Fibrinolytic Activity. Arch. Biochem. Biophys., **40**, 346, 1952.
6) Bigelow, W. G., Lindray, W. K. & Greenwood, W. F.: Hypothermia-Its Possible Role in Cardiac Surgery. Ann. Surg., **132**, 849, 1950.
7) Biggs, G. & Macfarlane, R. G.: Human Blood Coagulation and Its Disorders. Blackwell, Oxford 1953.
8) Brown, I. W., Jr., Smith, W. W. & Emmons, W. O.: An Efficient Blood Heat-Exchanger for Use With Extracorporeal Circulation. Surgery, **44**, 372, 1958.
9) Bunker, J. P. & Goldstein, R.: Coagulation During Hypothermia in Man. Proc. Soc. Exp. Biol. Med., **97**, 199, 1958.
10) De Nicola, P.: Thrombelastography. Charles Thomas, Springfield, Ill., 1957.
11) Dieckhoff, J.: Zur Behandlung der Säuglingsintoxikation mit Pereston N. Dtsch. med. Wschr., **77**, 1021, 1952.
12) Gollan, F.: Physiology of Cardiac Surgery. Hypothermia, Extracorporeal Circulation and Extra corporeal Cooling. Charles Thomas, Springfield, Ill., 1959.
13) Gollan, F., Grace, J. T., Schell, M. M., Tysinger, D. S. & Feaster, L. B.: Left Heart Surgery in Dogs during Respiratory and Cardiac Arrest at Body Temperature Below 10°C. Surg., **38**, 363, 1955.
14) Gordon, A. S., Meyer, B. W. & Jones, J. C.: Open-heart Surgery Using Deep Hypothermia Without an Oxygenator. J. Thor. Card. Surg., **40**, 787, 1960.
15) Hartert, H.: Die Thromboelastographie. Eine Methode zur physikalischen Analyse des Blutgerinn-

ungsvorganges. Ztschr. ges. exper. Med. **117**, 189, 1951.
16) Hikasa, Y. et al.: Significance of Fat Nutrition. I. Surgical Diagnosis and Treatment, **1**, 332, 1959.
17) Hikasa, Y. et al: Significance of Fat Nutrition. II. Ibid., **2**, 117, 1960.
18) Hikasa, Y. et al.: Significance of Fat Nutrition. III. Ibid., **2**, 253, 1960.
19) Hikasa, Y. et al.: Significance of Fat Nutrition. IV. Ibid., **2**, 386, 1960.
20) Hikasa, Y. et al.: Significance of Fat Nutrition, V. Ibid., **2**, 648, 1960.
21) Hikasa, Y. et al.: Significance of Fat Nutrition. VI. Ibid., **2**, 931, 1960.
22) Hibino, S.: Bleeding Diathesis. A Trend of Medicine, No. 13. Kanahara Shoten, Tokyo, 1957.
23) Hummel, K. und Halse, Th.; Theoretische Grundlagen der Blutstillenden Wirkung von Kolloiden. Klin. Wschr., **30**, 688, 1952.
24) Hyhus, L.: Discussions on Blood Changes in Extracorporal Circulation. In Allen ed.: Extracorporeal Circulation, p. 266, Springfield Ill., Charles Thomas, 1960.
25) Ida, Y.: Experimental Studies on Carbonhydrate Metabolism during Heart-lung Bypass, with Special Reference to a Comparison of Pulsatile Flow with Nonpulsatile Flow. Arch. Jap. Chirur. **31**, 1962.
26) Igami, H. et al.. Effect of ε-aminocaproic acid upon the Abnormalities of Blood Components. Internal Medicine & Pediatrics. (Japan) **15**, 648, 1960.
27) Inoue, Y.: Basic Study Concerning to Blood Canals on Artificial Heart-Lung Apparatus. I. Blood Coagulation Control by Mean of Silicone Tratment of Glass Tubing. Respiration & Circulation. (Japan) **1**, 121, 1953.
28) Kirby, C. K.: Discussions on Blood Changes in Extracorporeal Circulation. In Allen ed.: Extracorporeal Circulation. Charles Thomas, Springfield, Ill. 1960.
29) Kirklin, J. W. et al.: Studies in Extracorporeal Circulation. I. Applicability of Gibbon Type Pump Oxygenator to Human Intracardiac Surgery: 40 Cases. Ann. Surg. **144**, 2, 1956.
30) Kirklin, J. W., Patrick, R. T. & Theye, R. A.. Theory and Practice in Use of a Pump Oxygenator for Open Intracardiac Surgery. Thorax, **12**, 93, 1597.
31) Kuroyanagi, T.: Fibrinolysin. Igakushoin, Tokyo, 1954.
32) Kwaan, H. C.: & Mcfadzean, A. J. S.: Plasma Fibrinolytic Activity Induced by Ischaemia. Clin. Sci., **15**, 245, 1956.
33) Lillehei, C. W., R. A. Dewall, R. C. Read, H. E. Warden and R. L. Varco.: Direct Vision Intracardiac Surgery in Man Using a Simple Disposable Artficial Oxygenator. Dis. Chest., **24**, 1, 1956.
34) Lincolon, A. F., A. J. Moorman and R. L. schultz : Fibrinolysis Following Thoracic Surgery. Surg. Gynec. & Obst., **105**, 541, 1958.
35) Matsuoka, M.: Clinical Pathology of Blood Coagulation., Acta. Haem. Jap., **19**, 495, 1956.
36) Macfarlane, R. C. & R. Biggs: Observations on Fibrinolysis. Spontaneous Activity Associated with Surgical Operation, Trauma, etc., Lancet **2**, 862, 1946.
37) Miyazaki, Y. et al.: On the Abnormal Blood Coagulation Induced by Surgical Stressor. J. J. S. S., **60**, 632, 1959.
38) Mole, R. H.: Fibrinolysis and the Fluidity of the Blood Post-mortem. J. Path. Bact., **60**, 413, 1948.
39) Morita, H.: Platelets and Bleeding Diathesis. Nihonrinsho (Japan) **17**, 2103, 1959.
40) Motoyama, N.: Biochemical Studies on Blood Undergoing Extracorporeal Circulation with a Pump-oxygenator. J. J. A. T. S., **9**, 961, 1961.
41) Mullertz, S. & Lassen, M.: An Activator System in Blood Indispensable for Formation of Plasmin by Streptokinase. Proc. Soci. Exp. Bio. Med., **82**, 261, 1953.
42) Mullertz, S.: A Plasminogen Activator in Spontaneously Active Human Blood. Proc. Soci. Exp. Bio. Med., **82**, 291, 1953.
43) Nonoyama, A.: Hemodynamic Studies on Extracorporeal Circulation with Pulsatile and Nonpulsatile Blood Flow. Arch. Jap. Chirur., **29**, 1381, 1960.
44) Nygaard, K. K.: Hemorrhagic Diseases: Photo-Electric Study of Blood Coagulability. St. Lois C. V. Mosby, 1941.
45) Ogata, T. et al.: A Comparative Study on the Effectiveness of Pulsatile and Nonpulsatile Blood Flow in Extracorporeal Circulation. Arch. Jap. Chirur., **29**, 59, 1960.
46) Okamoto, S. & Tsukamoto, Y.: Studies on the Fibrinolysis; Its Relation to the Anaphylaxis and the Inflammation. J. Keio Med. Soci., **28**, 295, 1953.

47) Okamoto, S. et al.: A Suppressing Effect of ε-amino-N-caproic acid on the Bleeding of Dogs, Produced with the Activation of Plasmin in the Circulatory Blood. Keio J. Med., **8**, 247, 1959.
48) Perkins, H. A., Osborn, J. J. & Gerbode, F.: A Simple Technique for the Comparison of Clotting Times of Fresh Whole Blood Exposed to Various Foreign Surfaces. J. Card. Vas. Surg., **1**, 180, 1960.
49) Perkins, H. A.; Problems in Coagulation. In Allen ed.: Extracorporeal Circulation. Springfield Ill., Charles Thomas, 1960.
50) Reppe, W.: Neue Entwicklungen auf dem Gebiet der Chemie des Acetylens und Kohlenoxyds. Springer Verlag, Berlin-Göttingen-Heiderberg, 1949.
51) Ruhe, C. H. W. & Horn, R. H.: Circulatory and Respiratory Effects of Hypothermia Induced by Blood Refrigation. Am. J. Physiol., **182**, 325, 1955.
52) Sato, A.: Studies on Fibrinolysis II. On the Inhibitory Action of ε-amino-N-caproic acid Upon Fibrinolysis. Nisshinigaku (Japan), **46**, 161, 1959.
53) Schubert, R.: Die Anwendung von Periston N zur Serum und Zellwäsche und ihre klinische Bedeutung. Dtsch. med. Wschr., **47**, 1847, 1951.
54) Sherry, S., A. P. Fletcher & N. Alkjaersig: Fibrinolysis and Fibrinolytic Activity in Man. Physiol. Reviews, **39**, 343, 1959.
55) Shirotani, H. et al.: An Appraisal of Essential Fatty Acid in Hypothermia. I. Fundamental Experiments. J. J. Anes., **10**, 8, 1961.
56) Shirotani, H. et al.: An Appraisal of Essential Fatty Acid in Hypothermia. II. Clinical Application. J. J. Anes., **10**, 92, 1951.
57) Swan, H.: Surgery by Direct Vision in the Open Heart during Hypothermia. J. A. M. A., **153**, 1081, 1953.
58) Takeda, J.: Experimental Study on Peripheral Circulation During Extracorporeal Circulation, with a Special Reference to a Comparison of Pulsatile Flow with Nonpulsatile Blood Flow. Arch. Jap. Chirur. **29**, 1407, 1960.
59) Tatsuda, N.: Experimental Studies on Extracorporeal Circulation with Artificial Heart Lung Apparatus, With Special Reference to Improvement of the Circuit and Apparatus and to Histological Investigation. Arch, Jap. Chirur. **31**, 1962.
60) Taguchi, K. et al.: Studies on the Deliverate Postoperative Care of Extracorporeal Circulation Utilizing Pump-oxygenator. Preliminary Report: Postoperative Protein Metabolism and Its Adequate Managements. Jap. J. thor. Surg., **14**, 25, 1961.
61) Troll, W. & Sherry, S.: The Activation of Human Plasminogen by Streptokinase J. Biol. Chem., **213**, 881, 1955.
62) Tsuchihashi, K.: Experimental Studies on the General Hypothermia for the Open Heart Surgery. J. J. A. T. S., **7**, 809, 1959.
63) Tsuchiya, K.: Behavior of Fibrinolysin in the Banked Blood. J. Keio Med. Soc., **34**, 191, 1957.
64) Ungar, G. & H. Hayashi: Enzymatic Mechanism in Allergy. Ann. Allergy. **16**, 542, 1958.
65) Virchow, R.: Die Cellularpathologie. (4th ed.) Berlin, 1871.
66) von Kaulla, K. N. & M. Weiner: Studies of Coagulation and Fibrinolysis by New Technic of Continuous Recording. Blood, **10**, 362, 1956.
67) von Kaulla, K. N. & H. Swan: Clotting Deviations in Man During Cardiac Bypass: Fibrinolysis and Circulating Anticoagulant. J. thor. Surg., **36**, 519, 1958.
68) von Kaulla, K. N & H. Swan: Clotting Deviations in Man Associated With Open-heart Surgery During Hypothermia. J. thor. Surg., **36**, 857, 1958.
69) Watanabe, H.: Studies on the Hemorrrhage and Exudation Associated With the Experimental Activation of Plasmin System in vivo. J. Physiol. Soc. Jap., **22**, 983, 1960.
70) von Kaulla, K. N.: Intravenous Protein-free Pyrogen-A Most Powerful Fibrinolytic Agent in Man. Circulation, **17**, 187, 1958.
71) Wesolowski, S.: Discussions on Blood Changes in Extracorporeal Circulation In Allen ed.: Extracorporeal Circulation, p 271, Springfield, Ill., Charles Thomas, 1960.
72) Wesse, H.: Indifferente Kolloide in Chirurgie und innere Medizin. Dtsch. med. Wschr., **76**, 757, 1951.
73) Wesse, H. und W. Scholtan: Pharmakologie des Pereston-N-. Dtsch. med. Wschr., **76**, 1492, 1951.

和文抄録

体外循環時における出血傾向についての実験的研究

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体外循環時における最も不快な合併症の一つとして出血傾向があげられているが，われわれの研究室で試作した脈動式ポンプ並びに Waud-Salisbury 型酸化装置を使用して体外循環実験を行つたところ，術中術後の出血傾向による胸腔内出血の結果試獣はすべて斃死した．それでこのような出血傾向は，体外循環によつて血液凝固過程の何れかの相が障害されたためではないかと考えられたので，血液凝固の各相を第4相の線維素溶解過程にいたるまで追求すると同時に，Priming blood の採血法及び体外循環回路の改良等を重ねた．

当初の実験では酸化装置に大量のフイブリンが析出して血中フイブリノーゲン量は100mg% 前後と明らかに減少していた．そこでまず血液の損傷を出来る限り少くする意味で Primingblood を Silicone-Coating した採血瓶に採取して10分間に亘る部分灌流実験を行つたら，著明な出血傾向は消失して試獣は生存し得るようになつた．併し Waud 型酸化装置を使用して完全体外循環を行うと，試獣は依然胸腔内出血によつて斃死した．そこで基礎実験として，Coating を行つた採血瓶に anti-plasmin 製剤である Ipsilon 及び Plasma expander の一種である P. V. P. を加えて採取した血液と，Coating を行つていない採血瓶に Ipsilon 等を加えずに採取した対照血液について，脈動式ポンプ及び Sigma motor ポンプを用いて，連結回路の材質及び気泡抜き等を検討しながら，これら二種の血液の回転後の溶血度，フイブリノーゲン量を測定した．その結果前処置を加えた血液は非処置血に比べて，いずれの場合でも損傷が少なかつたが，連結回路を Silicone rubber となして気泡抜きも Coating を行い且つそれを回路内に一個だけ挿入した場合に最も良い成績を得られた．なお二つのポンプの間では差を見出し得なかつた．この結果から，前処置を行つた Priming blood で，Waud 型酸化装置に代るに日本血液研究所製の二重円筒型酸化装置を以てして体外循環を行つたところ，術中術後の出血傾向は全く見られなくなり，遮断時間も漸次延長出来て30分間の完全体外循環に於て技術的過誤の1例を除いて全例長期生存せしめ得た．そしてその際に次のような測定結果を得た．1）常温下体外循環群7例のうち3例に灌流後トロンボプラスチンの減少を認めた．2）プロトロンビン時間は，常温下灌流群及び急速冷却法群のいずれに於ても生理的範囲にとどまつたが，冷却法群では再加温時に稍々短縮する傾向がみられた．3）フイブリノーゲンは，当初の実験では酸化装置に大量に析出したために非常に減少したが，装置や回路を改良の結果灌流後のフイブリノーゲン減少は最大100mg%前後に止つた．4）トロンボエラストグラム：灌流前及び後に r, k, ma の著明な変動はみられず，急速冷却法時の軽度の低凝固性も再加温後には殆んど灌流前値に復した．5）線維素溶解能：Ipsilon 添加血液では，灌流後の線溶能の亢進はみられなかつたが，非添加血液で灌流した場合には，灌流後可成りの亢進がみられた．又 Streptokinase で賦活した線溶現象をトロンボエラストグラフで観察しても Ipsilon 添加血では特徴的な Pattern が得られないが，非添加血では線溶現象の増強がみられた．

従つて，実験の当初に見られた出血傾向は，人工心肺装置の血液との接触面及び採血法の不備によつて血液損傷並びにフイブリン析出が著しく惹起されたために，血小板減少や線溶能亢進等が招来されて二次的に毛細管透過性も著しく上昇し，その結果滲出性の出血を招くに至つたものと考えるのが妥当であろう．それ故に体外循環時に惹起され得る毛細管透過性の異常状態を是正する目的には Ipsilon 及び P. V. P. を Priming blood 中へ添加することはまことに有意義である．