EXPERIMENTAL STUDIES ON EXTRACORPOREAL CIRCULATION WITH ARTIFICIAL HEART-LUNG MACHINE, WITH SPECIAL REFERENCES TO IMPROVEMENTS OF THE CIRCUIT AND APPARATUS, AND TO HISTOLOGICAL INVESTIGATION

by

NORIKAZU TATSUTA

From the 2nd Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. YASUMASA AOYAGI)
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INTRODUCTION

The open heart operation for complicated heart diseases can be performed safely only when sufficient duration of cardiac arrest or heart lung by-pass is acquired. However, on this point there are many unsolved problems in the hypothermia and extracorporeal circulation.

Many investigations in this area have been performed in our laboratory. HIKASA[9,10,11,12,13,14], SHIROTANI[38,39], TOMIOKA[46], SAITO[32] and KUWANA[19] proposed that specific premedications were effective to cardiac function under hypothermia, and good results have been obtained clinically. On the other hand, OGATA[23,24] et al. produced a new type of pulsatile pump and TAKEDA[45], NONOYAMA[22], IDA[16] and SASAKI[35] emphasized the necessity of pulsatile flow during the extracorporeal circulation with this pump. And they published that pulsatile flow during the extracorporeal circulation was safer for body than non-pulsatile flow, especially when the flow rate is lesser than 100cc/kg min. or prolonged circulation over 30 minutes was requsted.

But SASAKI[35] noticed that this machine was not enough for clinical application, so we made present experiments aiming at getting survivors after perfusion.

Seeking after the cause of death, we have improved the instrument of the artificial heart-lung machine, in the course of these experiments.

The purpose of this paper is to show the improved points of the instrument and also describe the patho-histological findings using these circuits.

CHAPTER I. EXPERIMENTAL METHODS

Artificial heart : We used a pulsatile pump, although we used a sigma motor pump in the first stages of our experiments.

Artificial lung : At first, we used foam-oxygenator of WAUD-SALISBURY[33,34,49] type, then we improved this oxygenator and produced a form of the film oxygenator. However, later the bubble oxygenator of double cylinder type has been used which was designed by SAEGUSA[28] et al. of Tokyo University and made by Nippon Junkansōchi Kenkyusho.

Circuit : The circuit employed in this experiment is shown in Fig 1. There are

some differences between the case where the bubble oxygenator was used and other cases. Material of circuit was vinyl-chloride tube at the first stage, but later we used silicone gummi tube. And silicone coating was also employed for metal and glass portions.

Experimental animals: Mongrel dogs weighing 6～13 kg. were used.

Anesthesia: After intravenous injection of pentobarbital sodium (20～25mg/kg), trachea was immediately intubated with an endotracheal tube for the closed anesthesia with ether and pure oxygen.

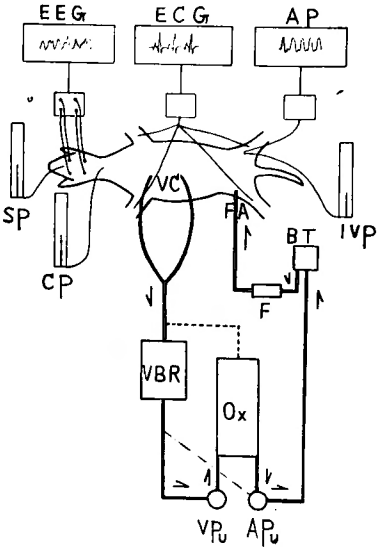
Priming blood: Heparinized blood (4 mg/dl) of 1,000～1,500 cc was taken from non-anesthetized donor dogs.

Later P.V.P. (polyvinylpyrrolidone), Ipsilon (antiplasmin drug) and A C.D. (acid citrate dextrose) solution were added to priming blood.

In the case with A C.D. solution, calcium gluconate was added to the perfusing blood before stop of perfusion. Perfusion was further continued for some time.

Cross-match-test between donor and recipient dogs was not performed.

Fig. 1 The perfusion circuit



—— with double cylinder oxygenator
····· with WAUD-SALISBURY type foam oxygenator, or our film oxygenator
– – – without oxygenator
AP arterial pressure, SP pressure of sagital sinus, CP : cerebrospinal pressure, IVP inferior caval vein pressure, VC : vena cava, VBR : venous blood reservoir, Ox : oxygenator, VPu : venous pump, APu : arterial pump, BT bubble trap, F : filter, FA : femoral artery

Desinfection: The inside of the circuit was washed with clean saline solution, but sterilization was not performed.

Autopsies: Autopsies were performed immediately after death in the dead cases, but in the survival cases, dogs were kept alive for 48 hours after perfusion. In all cases a detailed histological examination of the liver, spleen, kidney, adrenal gland, lung and brain was carried out. Hematoxilin-Eosin stain, Sudan III stain and NISSL's stain were performed.

Arterial blood pressure was measured by the STATHAM strain gauge electric manometer at femoral artery. Inferior caval vein pressure, and pressures of sagital sinus and cerebrospinal liquor were measured by means of polyethylene catheters which were induced to each portion and connected to water manometer.

The electrocardiographic lead II and occipitofrontal electroencephalographic lead were recorded.

CHAPTER II. COURSE OF EXPERIMENTS AND RESULTS

The process of experiment is divided into following groups:

Table 1 Partial perfusion without artificial lung

Exp. No.	Result	Time of death (hours)	Pump	Flow rate cc/kg./min.	Perfusion time (minutes)	Hemorrhagic diathesis	Cause of death	Improvement
4	died	15	pulsatile	15	10	++	hemorrhagic diathesis	—
5	died	12	sigma motor	20	10	+++	hemorrhagic diathesis filarial embolism	—
6	died	5	pulsatile	25	10	+++	hemorrhagic diathesis	—
7	survived		sigma motor	35	10	—	—	silicone coating
8	died	3	sigma motor	—	10	—	filarial embolism	silicone coating
9	survived		pulsatile	30	10	—	—	silicone coating

Group I: Partial perfusion

(A) Partial perfusion without artificial lung

(B) Partial perfusion with artificial lung

(C) Rapid cooling by A-A shunt

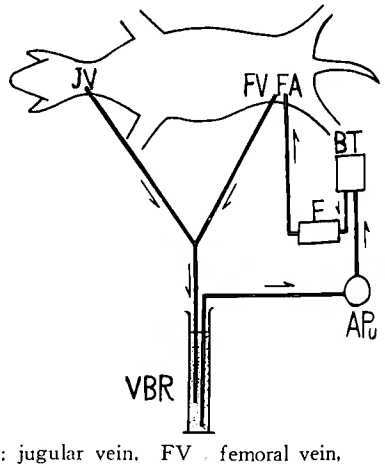
Group II: Total perfusion

(1) Group I. (A) (Table 1)

As we expected from OGATA[23,24], TAKEDA[45], NONOYAMA[22], IDA[16] and SASAKI's[35] papers, all animals died due to bleeding in the thorax and from the slight wound of nasal mucosa at the first stage of our experiment owing to the very strong hemorrhagic diathesis after perfusion. Two factors would be pointed out as the cause of such hemorrhagic diathesis, namely vascular factor and blood factor. At first we noticed blood factor, and then in order to solve that problem, we have performed the experiment of extracorporeal circulation without artificial lung in the circuit, which was suspected to be a large cause of blood destruction (Fig. 2).

In these cases, venous catheters were inserted from jugular vein and femoral vein to caval veins and thoracotomy was not done. However, hemorrhagic diathesis was found greatly after only 10 minutes perfusion and we could not rescue the animals from death by bleeding from the operation wound and slight wound which was made in the nasal mucosa at the time of inserting the cannula for oxygen supply after operation. Histologically, severe bleeding was found in the liver (Photo. 2), spleen (Photo. 7),

Fig. 2 The circuit of group IA (partial perfusion without oxygenator)



JV : jugular vein, FV : femoral vein, FA : femoral artery, VBR : venous blood reservoir, APu : arterial pump, BT : bubble trap, F : filter.

Fig. 3 E.E.G., E.C.G. and arterial pressure wave in the case of no. 6

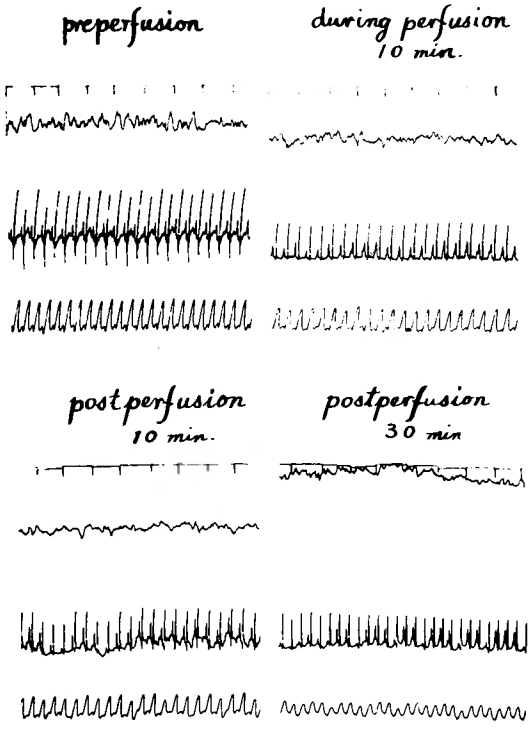
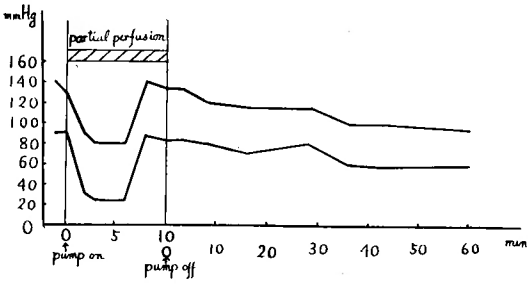


Fig. 4 Arterial pressure in the case of no. 6



kidney and lung (Photo. 20). In these cases, we noticed no change in E. C. G. and E. E. G. during perfusion, but animals died from severe shock after perfusion and low voltage was observed in E. C. G. and E. E. G. in this stage (Figs. 3 and 4).

No differences were seen in the result of pulsatile and sigma motor pump as the artificial heart.

From these facts we presumed that the hemorrhagic diathesis was caused by destruction of blood component due to materials of circuit or blood taking method from donor dogs. Following ABE's report[1], who was my coworker, silicone coating was performed on the reservoir and glass and metal portions of the circuit. After that, hemorrhagic diathesis disappeared and we succeeded in making dogs live long.

2) Group I. (B) (Table 2 and Fig. 5)

We performed partial perfusion with oxygenator of WAUD-SALISBURY type which had been used by OGATA et al. In these cases, hemorrhagic diathesis after perfusion was

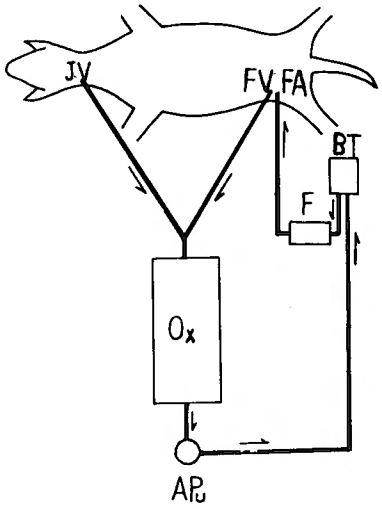
Table 2 Partial perfusion with artificial lung

Artificial lung	Exp. No.	Result	Time of death (hours)	Pump	Flow rate cc/kg./m.	Perfusion time (minutes)	Cause of death	improvement
Waud-Salisbury type	10	died	25	sigma motor	35	10	postperfusion shock	—
	11	survived	—	pulsatile	35	10	—	p. v. p. antilasmin drug
Our film oxygenator	12	survived		pulsatile	25	10	—	〃
	13	died	26	pulsatile	45	10	miliar infarction	〃
	14	survived	—	pulsatile	50	10	—	〃
Bubble type	15	survived	—	pulsatile	40	25	—	〃

In all cases of this group, silicone coating was employed.

not seen and the electrocardiographic and electroencephalographic findings were almost normal during perfusion, but we could not rescue the animal from the death by shock of unknown causes. Furthermore, the noticeable finding in these cases was great increase of secretion from nasopharyngeal region and this phenomenon was considered to be based on vagotonia due to intoxication. Great fibrin deposit was also found in the oxygenator at the contact site of blood and oxygen and at the same time the reduction of fibrinogen content in blood was notable [1]. From these results, we performed the same experiment in which Ipsilon (antiplasmin drug) and P.V.P. were added in the priming blood for the purpose of detoxicating the toxins derived from blood destruction in the artificial lung, and then we succeeded in keeping dogs live long.

Fig. 5 The circuit of group IB (using WAUD-SALISBURY type foam oxygenator or our film oxygenator)



JV jugular vein, FV femoral vein, FA femoral artery, Ox oxygenator, APu : arterial pump, F : filter, BT : bubble trap

The substances which cause vagotonia are yet unknown but they will be produced with blood destruction by bubbles. The blood of the preceding experiment remained a little in spite of powerful washing because such type of oxygenator are used repeatedly. We consider that the remaining blood is denatured into toxic substances. Therefore, we conclude that such type of oxygenator can not be used safely in the clinical operation.

To prevent the blood destruction by bubbles, screen or membrane oxygenator is considered to be adequate[4,7,17]. Then we manufactured a film oxygenator which was modified from WAUD-SALISBURY type of oxygenator (Fig. 6). Glass-balls in 2~3 cm diameter with silicone-coating were filled in the oxygenator as shown in Fig. 6. The venous blood drops down from the upper portion and produces the thin film of blood over the surface of glass balls. The oxygen blows into the oxygenator from the bottom. Oxygenation of blood is performed on the surface of glass balls. We succeded in getting the survivors by using this oxygenator However, from the viewpoint of the capacity of oxygenation, this oxygenator was inferior to the foam oxygenator of WAUD-SALISBURY type We were able to get the sufficient oxygenated blood in the series of experiment of partial perfusion under 300cc/min. of flow rate, but according to YAMAZAKI's[51] report, who was my coworker, it was impossible to get enough oxygenated blood in the series of experiment of total perfusion of group II with our film oxygenator in which flow rate was over 40/kg/min.

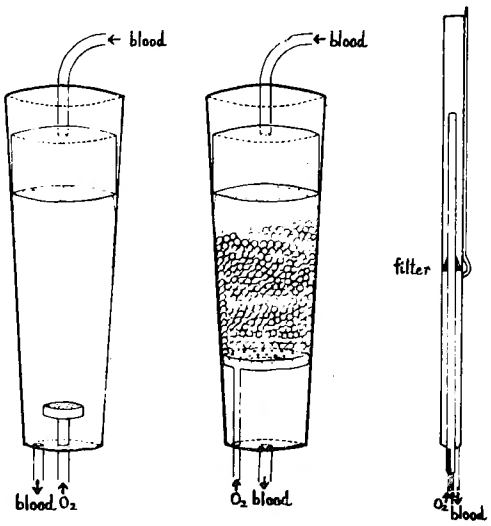
This type of oxygenator was thought to be impractical because very large instrument would be necessary in clinical cases. Furthermore, in spite of complete silicone coating on the glass balls a few fibrin clots were found, and infarct in the liver (Photo. 4) and

kidney (Photo. 12 and 13) was seen histologically in the case of no. 13 which was performed without filter. At that time we got the new oxygenator of bubble type which was designed by SAEGUSA et al. (Fig. 6). This oxygenator is small-sized and contains the filter in it, so does not require seperate debubbling chamber, and we are able to renew the oxygenator in every experiment.

In these experiments, blood destruction was lesser than with WAUD-SALISBURY type oxygenator. In the point of oxygenation, this type of oxygenator was superior to film oxygenator. But slight destruction of blood was unavoidable and production of fibrin clots by stirring the blood with bubble was also seen a little.

Since then we have used this type of oxygenator because this is thought to be best among the many types of oxygenator which we can get easily.

Fig. 6



left WAUD-SALISBURY type foam oxygenator
center our film oxygenator
right double cylinder bubble oxygenator (SAEGUSA et al.)

We succeeded in getting long term survivors in the experiment which was performed with this oxygenator for 25 minutes of partial perfusion. When we employed this oxygenator, we added a venous blood reservoir and venous pump to the circuit as illustrated in Fig. 8.

3) Group I. (C) (Table 3)

While we were studying the problem of oxygenator, we had a series of experiment of rapid cooling by arterio-arterial shunt. The purpose of this experiment was to eliminate the dangerous accident caused by oxygenator and to shorten the time of conventional surface cooling.

The pulsatile pump was used as artificial heart. Blood was taken out from femoral

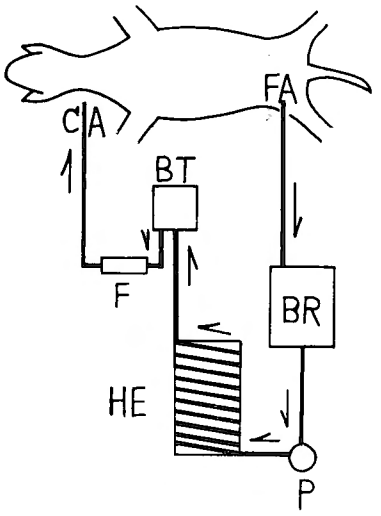
Table 3 Rapid hypothermia by A-A shunt

Exp. No.	Result	Time of death (hours)	Minimal E.T.(℃)	Minimal R.T. (℃)	Cooling time (minutes)	Rewarming time (minutes)	Perfusion time (minutes)	Cause of death	Inserting side of the arterial canule
16	died	2	18.5	24.5	33	87	120	cardiac failure	left
17	died	3	23.0	25.7	51	75	126	unknown	left
18	died	5	20.4	21.0	58	80	138	unknown	left
19	died	5	22.9	24.3	45	53	98	unknown	left
20	died	7	15.8	20.0	32	61	93	unknown	left
21	survived	—	22.0	24.5	24	41	65	—	right
22	survived	—	22.0	19.8	46	87	133	—	right

E. T. esophageal temperature
R. T. rectal temperature
In all cases of this group, p. v. p. and antiplasmin drug were added in the priming blood.

artery and infused into carotid artery. Heat exchanger was manufactured by us. It consisted of silicone gummi tube which was immersed in constant temperature bath (Fig. 7).

Fig. 7 The circuit of group IC (rapid hypothermia by A-A shunt)



CA : carotid artery, FA : femoral artery, BR : blood reservoir, P : pump, HE : heat exchanger, BT : bubble trap, F : filter.

As a premedication animals were given essential fatty acid, Vit. E. and dimethylaminoethanol by mouth during 5～7 days according to the method of HIKASA[9,10,11,12,13,14,19,32,38,39,46] et al., The results were shown in Table 3.

Effectiveness of this heat exchanger was inferior to metal heat exchanger which was reported by HARRISON-BROWN[3] and PIWNICA[26].

The time of cooling and rewarming was considerably long as a result of partial perfusion. At the time of cooling 2～5 minutes were needed to get down 1°C of body temperature, and 3～6 minutes to get up 1°C of body temperature.

Pumps are stopped for 10～15 minutes from the end of cooling to the beginning of rewarming. During that time pulsation fell to 30～40 per minute but heart never stopped. Ventriculotomy was not performed.

The lowest temperature was about 15 8°～23°C in esophagus and was 19 8°～25 7°C in rectum.

When animals were rewarmed to about 33°～36°C at esophageal temperature, perfusion was stopped and then surface rewarming was continued.

Neostigmin was injected when the esophageal temperature reached to 28°～30°C in the cooling phase, and promethazine was also injected after resuscitation. Perfusion time ranged from 65 to 138 minutes.

One animal died from cardiac failure. Many animals died relatively early after perfusion without awaking from anesthesia. The fall of activities of important organs such as brain, heart etc. was supposed to be the cause of death, but from the microscopic observations, it was impossible to find pathological changes in brain, heart and so on. E. C. G. returned back to normal. In these series of experiments, the noticeable fact was that two cases of long term survivors were inserted with the arterial cannula into right carotid artery, while all the dead cases were inserted into left side. In the hemodynamic view-point, we consider that right side delivery is reasonable for maintaining the perfusion quantity to brain, because, in left side delivery cases, the flow from the artery pump and that from heart meet and cancel each other, but in right side delivery cases do not. However, even in left side delivery cases, we could not find out any changes in the brain with microscopic observation. Therefore, we can not help considering that histological

Table 4 Total perfusion

Artificial lung	Exp. No.	Result	Time of death (hours)	Total perfusion time (minutes)	Flow rate cc/kg./m.	Cause of death	Improvement
Our film oxygenator	23	died	2	10	35	1) hemothorax 2) hyperthermia of blood	—
	24	died	3	10	35	hemothorax	—
	25	died	5	10	40	1) hemothorax 2) pulmonary edema	—
	26	died	0	10	35	technical failure	—
Bubble type (double cylinder)	27	died	5	10	60	hemothorax	—
	28	died	4	20	50	1) hemothorax 2) pulmonary edema	—
	29	died	2	20	70	hemothorax	—
	30	survived		15	45	—	1) electrocoagulation
	31	died	5	15	40	unknown	1) 〃
	32	died	2	15	45	insufficient transfusion	1) 〃
	33	survived		15	50	—	1) 〃
	34	died	12	15	40	1) hemothorax 2) filarial embolism	1) 〃
	35	died	12	15	40	filarial embolism	1) 〃
	36	survived		18	45	—	1) 〃 2) silicone coating of artificial lung
	37	survived		15	50	—	1) 〃 2) 〃
	39	survived		30	55	—	1) 〃 2) 〃
	41	survived		30	55	—	1) 〃 2) 〃
	42	survived		30	50	—	1) 〃 2) 〃
	43	died	3	30	50	technical failure	1) 〃 2) 〃
	44	survived		30	50	—	1) 〃 2) 〃 3) A.C.D. solution
	46	survived		30	55	—	1) 〃 2) 〃 3) 〃

In all cases of this group, admixture of p. v. p. and antiplasmin drug in the priming blood, and silicone coating of glass and metal parts of the circuit were employed.

change such as dissolution of NISSL bodies due to hypoxia does not yet appear immediately after perfusion.

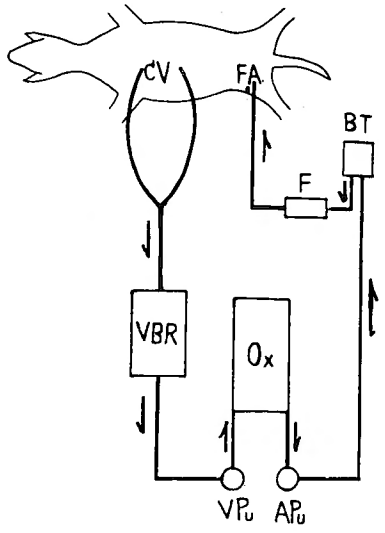
We succeeded in getting the long term survivors in the right side delivery experiments of rapid cooling by A-A shunt. But success or failure of this method depend on the cardiac function of the animals. Therefore, application to the operation of heart disease will be rather dangerous. Then we concluded that this method should not be applied clinically to the operation of heart disease. However, the safety of our pulsatile

pump was proved.

4) Group II. total perfusion (Table 4)

Circuit are shown in Fig. 8. The chest was entered through right 4th intercostal space, and after heparinization and cannulation, partial perfusion was performed for 2～3 minutes, and then caval veins were occluded and the venous return into heart was completely stopped. We had a few minutes' partial perfusion after ceasing the total perfusion and then stopped the pump and drew out the cannule. Intracardiac manipulations were not performed. In four experiments our film oxygenator was used, but in others we used bubble oxygenator mentioned above. Perfusion time was 10 minutes at first, then we extended the length of perfusion time to 15 minutes and 30 minutes. Results are shown in Table 4. Nine out of twenty one dogs survived. All animals which were perfused during 30 minutes survived except only one which died from serious technical failure. Almost all causes of death were due to the intrathoracic hemorrhage and filarial embolism at pulmonary valve.

Fig. 8 The circuit of group II (using double cylinder bubble oxygenator)



CV caval veins, FA femoral artery, VBR venous blood reservoir, VPu venous pump, APu : arterial pump, Ox : oxygenator, BT : bubble trap, F : filter

Coworker's ABE[1] mentioned that the causes of intrathoracic hemorrhage was not blood factor, but vascular factor. For the prevention of this bleeding we performed electrocoagulation and strict mass ligation of operation wound and then the death by severe bleeding after perfusion disappeared. Furthermore, we performed silicone coating on the inside of the artificial lung to prevent blood destruction since the experiment No. 36.

Since the experiment of No. 44, we have added A. C. D. solution into the priming blood to prevent the blood destruction during blood preservation. Thus we got 100% survival in the experiments of normothermic total perfusion under 30 minutes except one case of death through technical failure. We think that our new type machine of artificial heart-lung is applicable to clinical operation.

Experimental course in a typical case of total perfusion experiments during 30 minutes is shown in Figs 9, 10 and 11. In these experiments the maximal blood pressure ranged from 50 to 80 mm Hg. The inferior caval vein pressure and sagital sinus pressure were both 30～120 mm H O. Cerebrospinal pressure was 30～150 mm H O.

The electrocardiographic finding was greatly affected by body position and thoracotomy and so explaining of this finding must be deliberately done. But, in a few cases, P-increasing, S-T depression and T-inverse were seen during perfusion.

CLOWES mentioned that S-T depression and T-inverse on the electrocardiographic

Fig. 9 Experimental course of 30 minutes total body perfusion

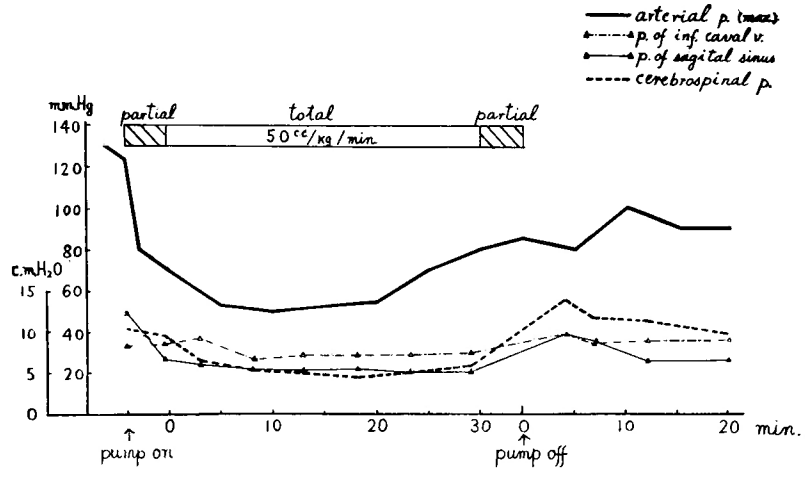


Fig. 10 E. E. G. in a case of 30 minutes total body perfusion

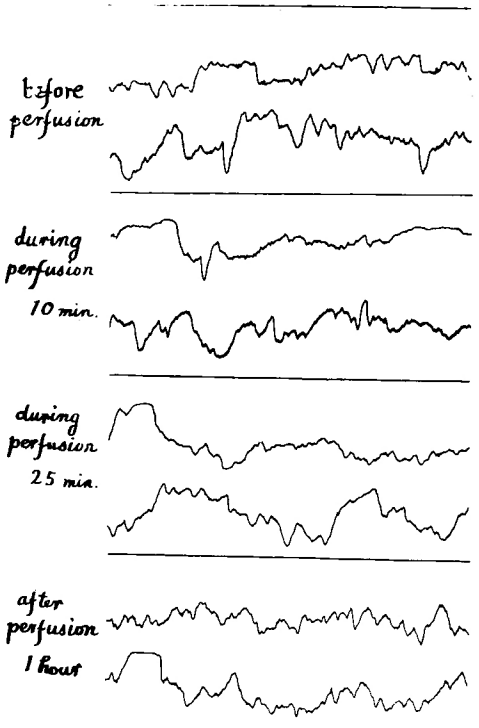
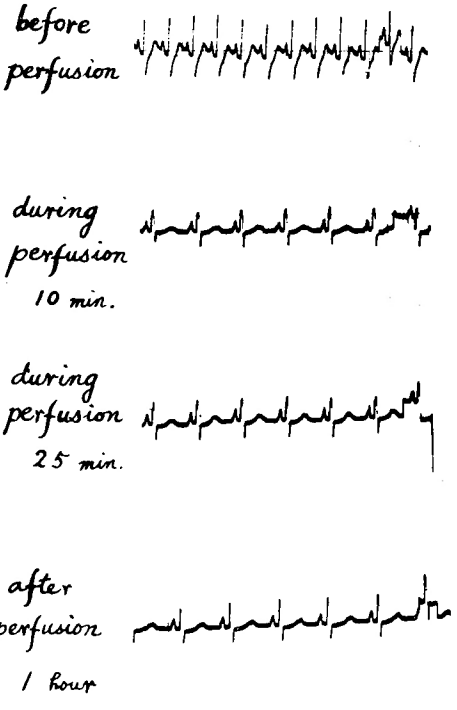


Fig. 11 E. C. G. in a case of 30 minutes total body perfusion



finding were noticed, if blood pressure went down under 60 mm Hg. We observed similar phenomena in our experiments, but many of these returned to normal with the lapse of time by keeping the blood pressure with the adequate transfusion after perfusion. Severe low voltage, arrythmia, and conduct disturbances could not be seen in the electrocardiographic finding. Bradycardia seems to be partly due to the fall of the temperature

of cardiac muscle by thoracotomy or extracorporeal circulation.

Sometimes, E. E. G. showed temporarily slow waves by the ether anesthesia. But, in almost all cases, E. E. G. showed no changes except in the case of severe unbalance of in-and out-flow. In the case of shock after perfusion slow waves and low voltage were observed.

HODGES[15] et al. mentioned that E. E G. is an accurate index of cerebral function and the brain is a sensitive mirror of abnomalities due to hypoxia, acidosis and other aberrations during total body perfusion.

CREECH[6] reported that E. E. G. will be affected by the change of arterial blood pressure or blood flow in brain. SAEGUSA[29,30] observed that E. E. G. was affected by change of blood pressure rather than blood flow in brain.

In any case, E. E. G. will be important as an indicator of cerebral function during and after perfusion. There were no abnormal changes on the physiological examination which was described previously, during the total perfusion under 30 minutes, which was our last experiment. It is compatible with the histological examinations described below.

CHAPTER III. SUMMARY OF EXPERIMENTAL COURSE

We have improved the artificial heart-lung apparatus used by T. OGATA[23,24], J. TAKEDA[45], A. NONOYAMA[22], Y. IDA[16] and H. SASAKI[25] in various aspects, and have gotten the good result in the survival experiments. The survival rate was 100% except the case of technical failure in the 30 minutes total perfusion. We have investigated the following problems in the course of these experiments

1) Blood taking method and materials of the circuit: Hemorrhagic diathesis which occurred in the first stage of our experiments disappeared by silicone coating of the glass and metal portions of the circuit, and by connecting the circuit with silicone gummi-tubes.

2) Admixtures to the priming blood: For the purpose of detoxication of toxins produced by blood destruction in the artificial lung, polyvinylpyrrolidone solution, anti-plasmin preparation and acid-citrate dextrose solution were added to the priming blood. Good results were obtained from this treatment.

3) Artificial lung: Noticing that blood destruction was great in the experiments with the artificial lung of WAUD-SALISBURY[33,34,49] type, we made a new type of film oxygenator but could not get satisfactory result. So, in the later experiments we used the bubble oxygenator made by SAEGUSA[28] and his coworkers, because this type of oxygenator was exchangeable with new one in every experiments.

4) Rapid hypothermia by A-A shunt: It was concluded from our experiments that this hypothermic procedure was not to be applied clinically to the operation of the heart disease. However, our pulsatile pump was proved to be safe in the long time perfusion. Besides, it is interesting from the view-point of hemodynamics that the animals cannulated with arterial cannula into the right carotid artery all survived, although the animals cannulated into the left side all died in the earlier period of postperfusion.

5) Hemostatic method: In the experiments of total perfusion accompanying thoracotomy, animals died from hemothorax in spite of the absence of coagulation failure in the earlier stage. This was prevented by electrocoagulation and strict mass ligation.

Table 5 Liver

Group	Experiment no.	Hemorrhage: Central vein	Hemorrhage: Glisson's sheath	Congestion: Central vein	Congestion: Glisson's sheath	Sinusoides	Infarction	Hemosiderosis	Compressed hepatic cell plate	Necrosis	Vacuole degeneration	Time length from perfusion end to autopsy (hours)	
Group IA	4	−	++	++	++	++	−	−	±	−	++	15	dead
	5	−	++	+++	++	++	−	−	−	−	++	12	
	6	−	++	+	++	++	−	−	+	−	++	5	
	7	−	−	−	−	+	−	−	−	−	+	48	survived
	9	−	−	−	−	+	−	−	−	−	−	48	
Group IB	10	++	−	++	+	++	−	−	++	−	++	25	dead
	13	+	−	++	−	++	+	−	++	+	++	26	
	11	−	−	−	−	+	−	−	−	−	−	48	survived
	12	±	−	++	+	−	−	−	−	−	+	48	
	14	−	−	+++	−	++	−	−	−	−	±	48	
	15	−	−	++	+	+	−	−	−	−	−	48	
Group IC	16	++	−	++	++	++	−	+	++	−	++	2	dead
	17	−	−	++	+++	++	−	−	−	−	+	3	
	18	−	−	++	++	++	−	−	−	−	−	5	
	19	±	−	++	++	++	−	+	−	−	++	5	
	20	−	−	++	++	++	−	−	−	−	±	7	
	21	−	−	−	−	−	−	−	−	−	−	48	survived
	22	−	−	−	−	±	−	−	−	−	±	48	
Group II	23	++	+	++	++	++	−	−	++	−	++	2	dead
	24	−	−	++	−	++	−	−	−	−	++	3	
	25	−	−	++	−	++	−	−	−	−	++	5	
	26	−	−	++	−	++	−	−	−	−	++	0	
	31	−	−	++	−	++	−	−	−	−	++	5	
	32	−	−	++	−	++	−	−	−	−	++	2	
	35	++	−	+++	++	++	−	−	++	−	++	12	
	43	++	−	++	++	++	−	−	+	−	++	3	
	30	−	−	++	++	++	−	−	−	−	−	48	survived
	33	−	−	++	++	++	−	−	−	−	+	48	
	36	−	−	++	++	++	−	−	−	−	−	48	
	37	−	−	++	++	++	−	−	−	−	+	48	
	39	−	−	++	++	++	−	+	−	−	−	192	
	42	−	−	++	++	++	−	−	−	−	−	96	
	44	−	−	++	+	++	−	−	−	−	−	48	

Table 6 Spleen

Group	Experiment no.	Hemorrhage	Congestion	Follicle: Atrophy	Follicle: Hypertrophy	Infected spleen	Increase of reticulum cells	Hemosiderosis	Time length from perfusion end to autopsy (hours)	
Group IA	4	−	++	++	−	−	−	−	15	dead
	5	++	+++	++	−	−	−	−	12	
	6	++	+++	++	−	−	−	−	5	
	7	−	−	−	−	+	+	−	48	survived
	9	−	++	−	+	−	−	−	48	
Group IB	10	−	++	−	−	−	+	−	25	dead
	13	−	+	++	−	−	+	−	26	
	11	−	++	−	+	−	−	−	48	survived
	12	−	−	−	−	+	−	−	48	
	14	−	+	+	−	−	++	−	48	
	15	−	−	−	−	−	−	−	48	
Group IC	16	−	++	−	−	±	−	−	2	dead
	17	−	±±	−	−	−	−	−	3	
	18	−	+	++	−	−	−	−	5	
	19	−	+	−	−	++	−	−	5	
	20	−	−	−	+	++	+	−	7	
	21	−	±	−	+	+	−	−	48	survived
	22	−	++	−	+	−	−	−	48	
Group II	23	−	++	+	−	−	−	−	2	dead
	24	−	++	+	−	−	−	−	3	
	25	−	++	−	+	−	−	−	5	
	26	−	+	++	−	−	−	−	0	
	31	−	++	−	−	−	+	−	5	
	32	−	++	−	−	−	−	−	2	
	35	−	+	−	−	−	−	++	12	
	43	−	++	−	−	−	−	+	3	
	30	−	+	−	−	−	−	+	48	survived
	33	−	±	−	−	+	−	+	48	
	36	−	−	−	−	−	−	++	48	
	37	−	±	−	−	−	−	−	48	
	42	−	−	−	+	−	−	−	96	
	44	−	−	−	−	−	+	−	48	

CHAPTER IV. HISTOLOGICAL FINDINGS

Liver (Table 5) : The most eminent changes observed were the circulatory disturbances which were histologically expressed mainly as acute congestive picture which was

most distinct in the liver among all the organs investigated. That is, the dilatation of hepatic veins and sinusoides with a gradient from the central vein toward the lobule and portal area was usually found. In severe cases, compressed basophilic hepatic cell plates and intracellular vacuoles were observed in the central area of lobule, and in the most severe cases central hemorrhage was observed, accompanying the rupture of central veins.

SHŌTU[40] described similar pictures in his experiments of the extracorporeal circulation.

On the other hand, it was expressed by SURUGA[44] that these changes were also observed in the experimental surgical shock. And there has been much argument about the relation between the changes observed in the liver of cardiac congestion and those of circulatory shock[27,42]. In our experiments some animals died from severe cardiac congestion, and others from circulatory shock, and in both cases, congestive picture was observed histologically. In order to show these facts, the author would like to discuss more fully the hemorrhagic cases.

Severe circulatory shock which was caused by the toxin produced in the artificial lung (WAUD-SALISBURY type) was the cause of death in the animal of No. 10 (Photo. 1). From acute cardiac failure the animal died two hours after perfusion (rapid hypothermia by A-A shunt) in the case of No. 16. Circulatory shock which resulted from blood destruction by hyperthermia over 45°C in the blood temperature regulator, was the cause of death in the case of no. 23. Severe congestion was produced by filarial embolism in the pulmonary ostium in the case of No. 35 (Photo. 5). In the case of No. 43 marked unbalance between in-and out-flow which was caused by falling of the venous cannula and returded reconstruction of extracorporeal circulation, was followed by severe circulatory disturbance.

On the facts above mentioned, each case had its cause of severe circulatory disturbances which were fatal to the animals. So, it might be concluded that the central hemorrhage which was observed histologically in these cases was the proof of severity of the circulatory disturbances.

However, hemorrhages without severe congestive changes were observed in some cases. Central congestive picture was not so intense and rupture of central veins was not proved in the cases of No. 4, No. 5 and No. 6, although hemorrhage was observed in the liver. In these cases hemorrhage was not found in the central area, but only in the Glisson's sheath. SASAKI[35] also described the same finding in his investigation. In these cases severe hemorrhage was proved in other organs, namely spleen (Photo. 7), kidney and lung (Photo. 20), so it might be considered that hemorrhagic diathesis which was produced by blood destruction in the blood taking bottle and extracorporeal circuit, caused hemorrhage in these cases, though it was difficult to explain why hemorrhage was observed only in the Glisson's sheath.

The experiment No. 13 was the case of miliary infarction caused by fibrin clot embolism (Photo. 4). In this case, bleeding was observed in miliary necrotic foci and in its neighbouring area, and hemorrhagic diathesis and severe circulatory disturbances were not seen clinically. Macroscopic infarction of the kidney accompanying hemorrhage was observed in the same case (Photo. 12 and 13).

Since there were found only a few erythrocytes in the intercellular space in the cases

of No. 12 and No. 19, hemorrhage might not have serious significance in these cases.

Moderate or marked congestive pictures without hemorrhage were always found in the dead cases, more markedly in the hypothermic group. These changes, it might be considered, were produced from various causes during various periods of postperfusion, but did not always bring fatal effects on the animals.

Judging from the fact that even in survivor cases, slight congestive changes were often observed, circulatory disturbances owing to the extracorporeal circulation were probably produced in all cases during postperfusion periods with various degrees Other factors, namely anoxia, bleeding from wounds, lung complication and so on were added to these disturbances: some of the animals endured, it might be supposed, while others succumbed to circulatory disturbances or the added factors, so that postoperative treatment of these circulatory disturbances had a great significance to the survivor of animals.

Compressed basophilic hepatic cell plate and intracellular vacuoles were both observed in the central area of lobule, and they ran parallel with severity of the circulatory disturbances. Intracellular vacuoles (Photo. 3) might be identical with hydropic or watery degeneration, which was described by some authors to be caused by anoxia, intoxication or rise of sinusoidal pressure and disappeared rapily when environment recovered normal [2,47]. In the survivor cases, these vacuoles were not found.

Hemorrhagic necrosis were observed in the central area of lobule of the case No. 35 (Photo. 5), which died from severe congestion caused by filarial embolism in the pulmonary ostium.

In the case of No. 13, miliary necrotic foci due to small emboli were scattered in the liver parenchym (Photo. 4), and in the cytoplasms of neighbouring area of these foci were proved lipoid granules by Sudan III stain. Large macroscopic infarction in the kidney was observed in the same case, but evidence of infarction in other organs, namely brain, heart, and lung was not proved probably because of partial perfusion in which the pump sent the blood mainly into the abdominal organs. The material of emboli was probably fibrin clot which was not proved histologically but found in the artificial lung (our film oxygenator). The filter in arterial line of extracorporeal circulation was not used in this case. Hemosiderosis due to hemolysis was proved only in several cases but it was not considered to be so serious.

Eventually, main findings in the liver were hemorrhage, congestive picture, infarction, and cellular degeneration, namely compressed hepatic cell plate and intracellular vacuoles. Many of the causes of these changes were removed by our improvement of the circuit, blood taking method and perfusion technic, so in the recent experiments histological changes became slight and small, and even these changes were all reversible (Photo. 6).

Spleen (Table 6) : Histological findings in the spleen were mainly circulatory disturbances as in the liver, but those severity was slightly milder than the liver. Acute central congestion with dilatation of sinusoides[20] was generally observed. Severe congestion and hemorrhage were also observed in the case of No. 35, which died from filarial embolism. Slight atrophy of splenic follicles was observed in some of the dead cases. Splenitis accompanying infiltration of polymorphnuclear leucocytes was observed in some cases, especially in the hypothermic group, but severity was generally slight in each cases

Table 7 Kideney

Group	Experiment no.	Hemorrhage } Border region between	Congestion } cortex and medulla	Hyperemia	Ischemia } Glomerulus	Cellular proliferation	Erythrocyte	Protein-like subst. } in Bowman's capsule	Cellular desquamation	Cloudy swelling	Granular degeneration } Tubulus	Hyaline degeneration	Enlarging of lumen	Protein-like substance	Eosinophilic cylinder } in tubulus	Erythrocyte	Infarction	Time length from perfusion end to autopsy (hours)	
Group IA	4	++	+	−	+	−	−	+	+	−	++	−	+	++	++	+	−	15	dead
	5	++	−	−	+	−	−	−	+	−	++	−	+	++	++	−	−	12	
	6	++	+	+	−	−	−	+	+	++	++	−	+	++	++	−	−	5	
	7	−	+	+	−	−	−	−	−	+	±	−	++	−	−	−	−	48	survived
	8	−	++	+	−	−	−	−	−	−	+	−	−	+	−	−	−	48	
Group IB	10	++	++	++	−	−	−	−	−	−	+	−	+	−	−	−	−	25	dead
	13	+	+	+	−	−	+	+	−	−	+	−	++	−	−	−	+	26	
	11	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−	−	48	survived
	12	−	+	±	−	−	−	−	+	−	−	−	++	−	−	−	−	48	
	14	−	+	+	−	−	−	−	−	−	−	−	++	−	−	−	−	48	
	15	−	+	+	−	+	−	−	−	−	+	−	++	−	−	−	−	48	
Group IC	16	−	+	−	−	−	−	+	+	+	−	−	++	+	−	−	−	2	dead
	17	−	−	−	−	−	−	−	+	−	+	−	++	−	−	−	−	3	
	18	−	−	−	−	−	−	−	++	+	+	−	++	+	−	−	−	5	
	19	−	+	−	−	+	−	−	−	−	+	−	−	−	−	−	−	5	
	20	−	−	−	−	±	−	−	−	−	+	+	−	−	−	−	−	7	
	21	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	−	48	survived
	22	−	+	−	−	−	−	+	−	−	+	−	++	−	−	−	−	48	
Group II	23	−	++	−	−	+	−	−	−	+	−	−	++	−	−	−	−	2	dead
	24	−	++	+	−	+	−	−	−	−	−	−	+	−	−	−	−	3	
	25	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	5	
	26	−	−	−	−	−	−	−	−	−	−	−	++	−	+	−	−	0	
	31	−	++	−	−	+	−	−	−	−	−	−	++	−	+	−	−	5	
	32	−	++	±	−	−	−	−	−	−	−	−	−	+	−	−	−	2	
	35	+	++	−	−	−	±	−	−	++	−	−	−	−	−	−	−	12	
	43	−	+	−	−	−	−	−	−	+	−	−	+	−	−	−	−	3	
	30	−	++	+	−	−	−	−	+	−	−	−	++	−	−	−	−	48	survived
	33	−	+	−	−	−	−	−	−	−	−	−	+	+	−	−	−	48	
	36	−	−	−	−	−	−	+	−	−	++	−	++	+	−	−	−	48	
	37	−	+	−	−	−	−	+	−	−	+	−	++	+	−	−	−	48	
	39	−	+	−	−	−	−	−	−	−	−	−	++	−	−	−	−	192	
	42	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	96	
	44	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	48	

(Photo. 8). Slight increase of reticulum cells was found in several cases, but its significance was not clear. Hemosiderosis was seen in some cases, especially in survivor cases, though it was slight (Photo. 9).

Kidney (Table 7) : The marked change in the kidney was also congestion which

was generally seen in the border region between the cortex and medulla, though its severity was more slight than that of the liver and spleen. Severity of congestion ran parallel with that of the liver, but in the hypothermic group congestion was not observed generally.

Hemorrhagic cases were almost coincident with those of the liver, but its severity was considerably moderate. Hemorrhage was observed in the cases of hemorrhagic diathesis (No. 4, 5 and 6), infarction (No. 13, Photos. 12 and 13), postperfusion shock (No. 10) and filarial embolism (No. 35, Photos. 10 and 11).

Changes of glomerulus and BOWMAN's capsule were generally moderate, and differences between the survivor and dead cases were not seen. Protein-like substances were observed in the capsular space and tubules in some cases. Erythrocytes in the capsular space were observed in the case of extreme congestion due to filarial embolism.

Cloudy swelling, granular degeneration and desquamation of the tubular epithelium were generally slight and probably reversible except the severe hemorrhagic cases. Degeneration of tubular cells of the lower nephrone and eosinophilic or protein-like cylinders in the tubules were more marked in the hemorrhagic case of group I (A). These findings might be concerned with the hemorrhagic diathesis caused by blood destruction.

Moderate degeneration of the tubular epithelium including one case of hyaline degeneration was observed in almost all cases of hypothermic group. These changes and ischemic pictures already mentioned were characteristic in this group.

In the case of No. 13, large macroscopic infarction in the shape of triangle was found. This was probably caused by fibrin clot embolism in the larger branch of renal artery (Photo. 12 and 13).

Although dilatation of tubular lumina was seen in many of both survivor and dead cases, it was considered as the complication following anesthesia and surgical operation.

Moreover, round cell infiltration was observed in some cases, but this was probably caused by parasites which were detected in one case.

Adrenal gland (Table 8) : Circulatory disturbances were slight in the specimen of hematoxilin eosin stain, and hemorrhage was not observed in any case.

Histological findings in the specimen of Sudan III stain were demonstrated in Table 8. Generally, these changes should be considered as stress-response[36]. The main histological change was the reduction of lipoid granules in each layer, especially in fascicular layer (Photo. 15). In the cases of marked lipoid reduction, large vacuoles were observed in the cell and the cell itself became larger than normal. Comparing the dead cases with the survivor, more marked lipoid reduction was found in the former, but more marked vacuoles existed in the latter. The border part between the glomerular and the fascicular layer was recognized clearly in the normal contrast animals and almost all dead cases, but became obscure in many survived cases owing to the proliferation of cell plate from the glomerular layer to the fascicular layer. Regeneration of the lipoid granules took place more widely in the cases of 4 and 8 days after perfusion than in the case of 2 days. The case of 8 days was almost normal histologically except some disarrangement of cell plates (Photo. 15 and 16).

In the hypothermic group the most characteristic picture was the minimal histological

Table 8 Adrenal gland

Group	Experiment no.	Hypertrophy	Atrophy	Decreas of lipoid granules	Intracellular vacuoles	Border-line between gl. layer and fascicular layer	Hypertrophy	Atrophy	Decreae of lipoid granules	Intracellular vacuoles	Hypertrophy	Atrophy	Decrease of lipoid granules	Intracellular vacuoles	Regenerative picture	Time length from perfusion end to autopsy (hours)	
		Glomerular layer					Fascicular layer				Reticular layer						
AI	9	−	−	+	−	clear	−	−	+	−	−	−	+	−	−	48	survived
Group IB	10	−	+	±	−	clear	−	−	##	−	−	−	+	−	−	25	dead
	13	−	−	##	−	not clear	−	+	###	##	−	−	##	+	−	26	
	11	−	−	±	−	not clear	+	−	###	##	−	−	##	−	−	48	survived
	12	−	−	±	−	not clear	+	−	##	+	−	−	±	−	+	48	
	14	−	−	+	−	clear	−	−	##	##	+	−	±	−	−	48	
	15	−	−	−	−	clear	−	−	##	−	−	−	±	−	−	48	
Group IC	16	−	−	−	−	clear	−	−	±	−	−	−	−	−	−	2	dead
	18	−	−	−	−	clear	−	−	+	−	−	−	±	−	−	5	
	19	−	−	−	−	clear	−	−	+	±	−	−	−	−	−	5	
	21	−	−	−	−	clear	−	−	+	+	−	−	−	−	−	48	survived
	22	−	−	−	−	clear	−	−	##	+	−	−	−	−	−	48	
Group II	23	−	−	−	−	clear	−	−	##	−	−	−	±	−	−	2	dead
	24	−	−	±	−	clear	−	+	+	−	−	+	+	−	−	3	
	25	−	−	+	−	clear	−	+	###	−	−	+	##	−	−	5	
	26	−	−	+	−	clear	−	##	###	−	−	+	##	−	−	0	
	31	−	−	−	−	clear	−	−	##	−	−	+	−	−	−	5	
	32	−	−	±	−	clear	−	+	±	−	−	+	±	−	−	2	
	35	−	−	##	−	clear	+	−	##	−	−	−	##	−	−	12	
	43	−	−	+	−	not clear	−	+	±	−	−	−	+	−	−	3	
	30	−	−	+	−	not clear	+	−	##	±	+	−	−	−	−	48	survived
	33	−	−	+	−	not clear	+	−	+	±	−	−	±	−	−	48	
	37	−	−	##	−	not clear	+	−	##	##	+	−	+	−	+	48	
	39	−	−	−	−	clear	−	+	±	+	−	−	±	−	−	192	
	42	−	−	+	−	not clear	−	−	+	−	−	−	±	−	+	96	
	44	−	−	##	−	not clear	−	−	##	−	−	−	+	−	+	48	

Table 9

Group	Experiment no.	Hemorrhage	Congestion	Degeneration of muscular cells	Time lenth from perfusion end to autopsy (hours)	
IA	7	−	−	−	48	survived
	9	+	+	−	48	
IB	10	−	+	±	25	dead
	13	−	−	−	26	
	11	−	−	−	48	survived
	12	−	−	−	48	
	14	−	−	−	48	
	15	−	−	−	48	
IC	16	−	+	±	2	dead
	17	−	−	−	3	
	18	−	−	−	5	
	19	−	−	−	5	
	20	−	−	−	7	
	21	−	−	−	48	survived
	22	−	−	−	48	
II	23	−	+	−	2	daed
	24	−	−	−	3	
	25	−	−	−	5	
	26	−	−	−	0	
	31	−	−	−	5	
	32	−	−	−	2	
	35	−	−	±	12	
	43	−	−	−	3	
	30	−	+	−	48	survived
	33	−	−	−	48	
	36	−	−	−	48	
	37	−	−	−	192	
	42	+	+	−	96	
	44	−	−	−	48	

change apparently distinguishable from the normothermic group (Photo. 17).

Heart (Table 9) : It has been described by some authors that the changes which are seen in anemic cardiac muscle, are often found at the subendocardial muscular layer

Table 10 Lung

Group	Experiment no.	Hemorrhage	Congestion	Exudate (in bronchioles)	Erythrocyte (in bronchioles)	Exudate (in alveoli)	Erythrocyte (in alveoli)	Thickening of alveolar wall	Emphysema	Atelectasis	Pneumonitis	Time length from perfusion end to autopsy (hours)	
Group IA	4	+	++	+	+	+	−	++	−	++	−	15	dead
	5	+	+	−	−	+	−	−	−	−	−	12	
	6	+++	+++	+	±	+	+	−	−	−	−	5	
	7	−	−	−	−	−	−	−	++	−	−	48	survived
	9	−	−	+	+	−	−	+	+	−	+	48	
Group IB	10	−	±	−	−	−	−	+	−	−	−	25	dead
	13	±	++	−	−	−	−	−	−	−	−	26	
	11	−	−	−	−	−	−	−	+	−	−	48	survived
	12	−	+	±	−	−	−	+	+	+	−	48	
	14	−	+	−	−	−	−	−	+	−	+	48	
	15	−	+	−	−	−	−	−	−	−	−	48	
Group CI	16	+	−	−	−	−	−	−	−	+	+	2	dead
	17	+	++	−	−	−	−	−	−	−	−	3	
	18	−	±	−	−	−	−	−	−	+	−	5	
	19	+	−	−	−	−	−	−	−	−	−	5	
	20	+	+	−	−	−	−	+	−	+	−	7	
	21	+	−	−	−	−	−	−	−	−	−	48	survived
	22	−	−	−	−	−	−	−	−	−	−	48	
Group II	23	−	+	−	−	−	−	+	−	−	+	2	dead
	24	−	+	−	−	−	−	+	−	+	−	3	
	25	+	++	+	+	+	+	+	−	−	+	5	
	26	+	++	−	+	−	+	−	+	−	−	0	
	31	−	+	−	−	−	−	+	−	−	+	5	
	32	+	+	+	−	−	+	+	−	−	+	2	
	35	+	++	+	−	−	−	±	−	−	−	12	
	43	−	+	+	−	−	−	+	−	−	+	3	
	30	−	+	+	−	−	−	+	+	−	++	48	survived
	33	−	−	−	−	−	−	+	−	−	+	48	
	36	−	−	−	−	−	−	−	−	−	−	48	
	37	−	−	−	−	−	−	−	−	−	−	48	
	39	−	−	−	−	−	−	+	−	−	+	192	
	42	−	+	−	−	−	−	+	−	−	+	96	
	44	−	−	−	−	−	−	+	−	−	+	48	

Table 11 Brain

(Degeneration of ganglion cells) (Nissl's stain)

Group	Experiment no.	Cerebral cortex	Ammon's horn	Cerebellar cortex	Time length from perfusion end to autopsy (hours)	
Group IA	4	−	−	±	15	daed
	5	−	±	−	12	
	6	−	−	−	5	
	7	−	−	−	48	survived
	9	−	−	−	48	
Group IB	10	−	−	−	25	daed
	13	−	−	−	26	
	11	−	−	−	48	survived
	12	+	±	+	48	
	14	−	−	−	48	
	15	−	−	−	48	
Group IC	16	−	−	±	2	daed
	17	−	−	±	3	
	18	−	−	−	5	
	19	−	−	±	5	
	20	−	−	+	7	
	21	−	−	−	48	survived
	22	−	−	±	48	
Group II	23	−	±	−	2	dead
	24	−	−	−	3	
	25	−	−	−	5	
	26	−	−	−	0	
	31	−	−	+	5	
	32	−	−	−	2	
	35	±	±	±	12	
	43	−	−	±	3	
	30	±	+	+	48	survived
	33	−	−	+	48	
	36	−	±	±	48	
	37	±	±	+	48	
	39	+	+	+	192	
	42	++	++	++	96	
	44	+	+	++	48	

of left ventricle, especially papillar muscle. Histological specimens were taken from the apical region of left and right ventricle in the present investigation. Results of the investigation were shown in Table 9. Except two cases with small hemorrhagic foci

(Photo. 18), slight congestion and minimal degeneration of heart muscle were found in some cases. Vacuole degeneration, myocardial fragmentation, edema of interstitium and round cell infiltration were all not detected. Eventually, the fatal lesions were not observed at all in the cardiac muscle.

Lung (Table 10) : Miscellaneous changes were found in the lung. The majority of these were probably arisen from ether anesthesia and thoracotomy. Congestion and moderate hemorrhage of many cases might be concerned with ether anesthesia[21], while local emphysema and atelectasis with thoracotomy.

Marked hemorrhage in the alveoli and bronchioles was observed in the case of No. 6 which showed hemorrhagic diathesis clinically (Photo. 20). Histological findings of pulmonary edema (Photo. 21) in the case of No. 25 coincided with the clinical picture.

At all, hemorrhagic diathesis and pulmonary edema were only caused by extracorporeal circulation and both changes disappeared in the last experiments.

Brain (Table 11) : It goes without saying that the brain is most important in the whole body. Furthermore, it is the most sensible organ to anoxia. The functional depression and the morphological lesion of brain caused by extracorporeal circulation have been studied by many investigators, and innumerable studies have been made on these problems from various points of view[8,25,31,37,41,43,48,50,52].

In our experiments, abnormal changes of electroencephalogram and cerebrospinal pressure were observed during and 1～2 hours after the extracorporeal circulation. In order to clarify the relation between clinical pictures and morphological changes, the author examined the brain of the animals histologically. Histological specimens were fixed in pure alcohol immediately after autopsy, then through the celloiden embedding were stained by hematoxilin eosin stain and NISSL's stain. The author mainly investigated circulatory disturbances, edema of white matter and degeneration of the ganglion cells of cerebral cortex, AMMON's horn and cerebellum.

As for the circulatory disturbances, slight congestive changes were only observed. Bleeding, red softening and white softening which were described by H. SAI[31] were not observed in our cases. Enlarging of VIRCHOW-ROBIN's space was observed in some cases, but it is not verified that these changes express the brain edema because of the lack of macroscopic findings of edema in each case. In short, brain edema with clinical significance was not found in our experiments.

Findings of NISSL's stained specimens were shown in Table 11. We must take a prudent attitude for the decision of degenerative changes of NISSL bodies because it is delicate and so must take considerable time for the occurrence of degenerative change of ganglion cells. In the present investigation, degenerative changes were observed in survivor cases rather than dead cases probably because of the above mentioned reason. However, degrees of degenerative changes were generally slight.

In the cases of 30 minutes perfusion, the changes were more severe in the case of 4 days after perfusion (Photo. 22, 23 and 24) than in the case of 2 days, but the changes were more slight in the case of 8 days than of the 4 days. By these findings it may be considered that these degenerative changes were reversible in the post-perfusion course.

CHAPTER V. SUMMARY OF HISTOLOGICAL FINDINGS

1) Circulatory disturbances: The most eminent changes throughout all investigated organs were the circulatory disturbances which were histologically expressed mainly as acute congestive picture. In cases of severe congestive picture, hemorrhage was observed in the liver, spleen and kidney. As the causes of these severe changes, failures of perfusion technic, filarial embolism in the pulmonary ostium, acute cardiac failure and circulatory shock due to the blood destruction were considered. The blood destruction was minimized by the improvement of the artificial lung, blood taking method and materials of extracorporeal circuit, so that circulatory shock caused by the blood destruction disappeared in the later experiments. Furthermore, hemorrhagic diathesis observed in the dead cases of group I. (A) disappeared by our improvements mentioned above.

2) Anoxic changes: The severity of anoxic changes ran almost parallel with that of circulatory disturbances in each case. Necrotic changes were observed only in the focus of infarction and in the site of severe hemorrhage. Anoxic changes in the brain were also reversible in the post-perfusion course.

3) Embolism: The embolism by fibrin clot, antifoam agent, air bubble and other foreign particles was written in many literature, but throughout the our experiments pictures of infarction in the liver and kidney were found in only one experiment without the filter in the circuit. Any infarction was not observed in the recent experiments with our improved circuit.

4) Hemolysis: In the histological investigation, hemolysis might be expressed as hemosiderosis and lower nephrone nephrosis. In the present investigation, hemosiderosis found in organs were all of slight degree, and degeneration of tubular cells as well as intratubular cylinder in the lower nephrone were found only in the dead cases of group I. (A). In the other cases, histological changes due to hemolysis were considerably slight.

5) Stress-response: Reduction of lipoid granules of the adrenal cortex were observed, however process of recovery from stress response was evident in the survivor cases.

6) Pulmonary complications: As for the complications due to the extracorporeal circulation, pulmonary edema was proved in one case, but none of them were observed in the recent cases.

7) Rapid hypothermia by A-A shunt: The most important finding is the scarcity of lipoid reduction in the adrenal cortex in this group.

SUMMARY AND CONCLUSION

We succeeded in the survival experiments of extracorporeal circulation using the pulsatile pump produced by OGATA and his co-workers, improving the circuit, artificial lung, blood taking method etc.

The survival rate in the 30 minutes complete perfusion was 100% with the exception of technical failure.

With the progress of experiments, findings of histology and other observations were improved gradually.

In the last stage of From our experiments, it was concluded that our artificial heart lung machine became usable and safe clinically from the view-point of histology.

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和文抄録

人工心肝装置を以てする体外循環の実験的研究 回路装置の改良と組織学的検索

京都大学医学部外科学教室第2講座 (指導：青柳安誠教授)
竜田憲和

複雑な必臓内手術を安全に行なうためには，十分な心血流遮断時間，或いは心停止時間が必要である．この点についてわれわれの教室の諸方，武田，野々山，井田，佐々木は脈動式ポンプを用いた体外循環実験を行ない，長時間の体外循環を行なう際には脈動流を用いた方が無脈動流を用いるよりも良好な結果が得られることを証明した．しかしこれらの実験はすべて生理実験であり動物の生存を目的とするものではなかつたので，この装置を臨床に応用するには尚可成りの改良を要することが予想されていた．

そこでわれわれは，この人工心肺装置を臨床的に安全に応用するために，更に動物の生存を指標として新しく器具改良に関して実験を行なつた．まず人工肺を用いない部分循環実験から始めてこれに成功した後，人工肺を用いた部分循環実験を行ない，更に完全体外循環実験へと前進した．尚この間に arterio-arterial shunt による急速冷却法の実験をも行なつた．この実験経過中に採血法，回路装置，循環装置等に種々の改良を加えたのであるが，その主な改良点は次のような点である．即ち

(1) 採血瓶及び回路内の金属及びガラス部に silicone coating を行ない，これを連結するビニール管を silicone rubber 管にかえることによつて血液因子による出血傾向を消失せしめ得た．

(2) 主として人工肺内部で生ずる血液破壊物質による中毒現象を防止するために polyvinyl pyrrolidone，抗プラスミン剤及び acid-citrate-dextrsse solution を血液中に添加して好成績を得た．

(3) 従来用いて来た Waud-Salisbury 型人工肺は血液破壊が著しいので，われわれは新しく一種の film oxrgenator を試作した．しかし満足し得る結果が得られなかつたので結局東京大学三枝らの設計による二重円筒気泡型の人工肺を採用した．

(4) また，胸腔内出血の一原因としてその開胸時の止血法に欠陥があつたので electrocoagulation 等を併せ行ないこれを可及的完全に行なうように努めた．

更に，a-a shunt による急速冷却実験では，臨床的にそれを応用することは出来ないとの結論に達したが，脈動式ポンプの安全性を証明することができた．

以上の実験経過を辿る間に，試獣の生存率は向上し遂に完全体外循環30分の実験群では，技術的過誤の1例を除けば100%の生存率を得るに至つた．

更に，この間の経過を主として病理組織学的に追求したが，その検索対象として肝，脾，腎，副腎，肺，心及び脳を選び，その際主要な所見として吟味したのは

(1) 循環障碍
(2) 無酸素症による退行変性
(3) 栓塞
(4) 溶血による変化
(5) ストレスに対する反応
(6) 肺合併症
(7) その他

である．そしてこの中で試獣の主死因となつた著明な所見は循環障碍のそれで出血死に次いでいる．而もこの変化は肝に最も著明に認められた．併しわれわれが前述のように諸種の点を改良することによつて，これらの組織学的所見も亦漸次改善され，われわれの最終段階の実験である30分間完全循環群では，変化はすべて軽微であり而も可逆性であることが判明した．従つてわれわれの人工心肺装置は最早臨床的にも安全に応用し得る段階に立ち至つたものと考えられる．

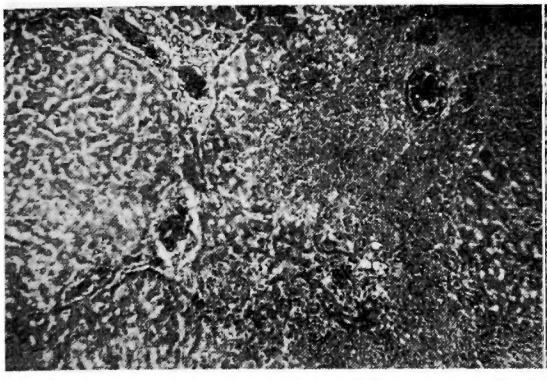

Photo. 1 (×60), Liver, No. 10 Severe central congestion accompanyig hemorrhage is observed.

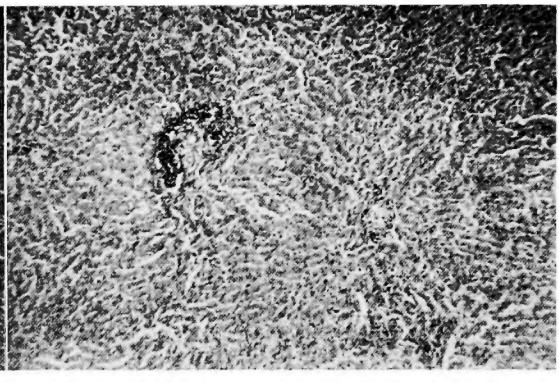

Photo. 2 (×60), Liver, No. 5 Hemorrhage is seen only in the GLISSON's sheath.

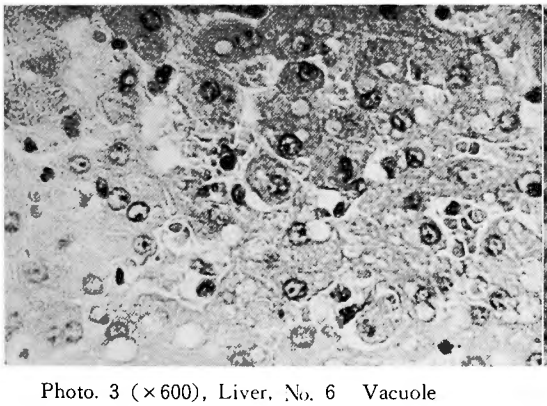

Photo. 3 (×600), Liver, No. 6 Vacuole degeneration is seen in the central area of lobule

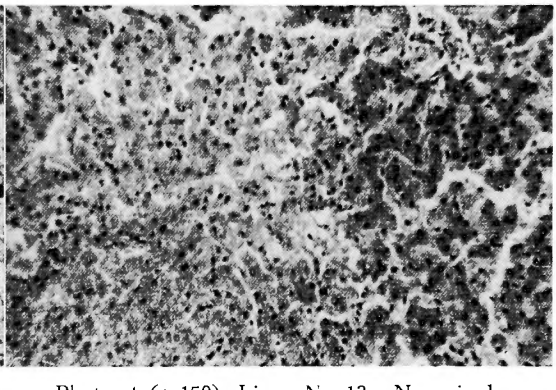

Photo. 4 (×150), Liver, No. 13 Necrosis due to miliar embolism is seen.

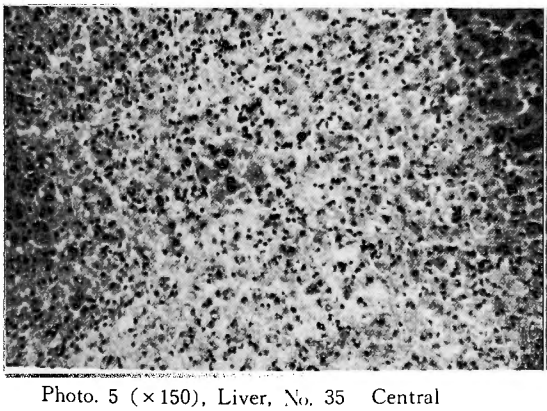

Photo. 5 (×150), Liver, No. 35 Central necrosis due to severe hemorrhage and congestion is observed.

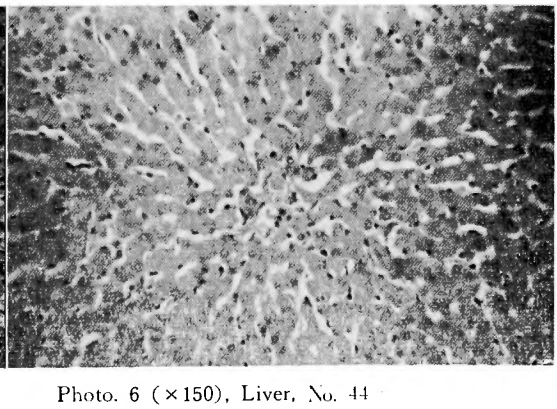

Photo. 6 (×150), Liver, No. 44 Histological change is minimal.

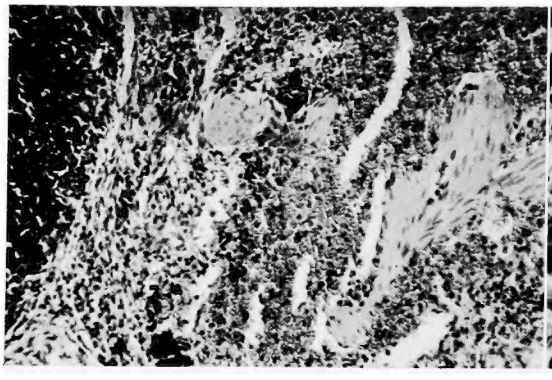

Photo. 7 (×150), Spleen, No. 6 Hemorrhage and severe hyperemia is seen in the splenic pulpa.

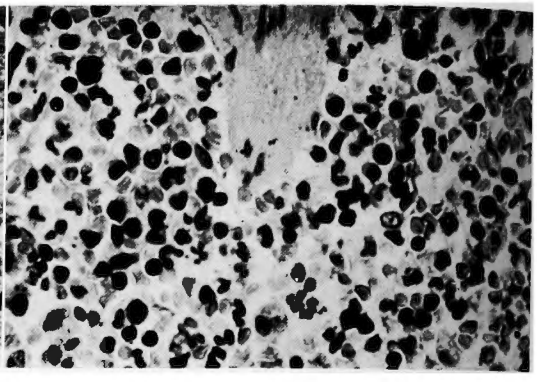

Photo. 8 (×600), Spleen No. 20 : Polymorphnuclear leucocytes are observed in the splenic pulpa.

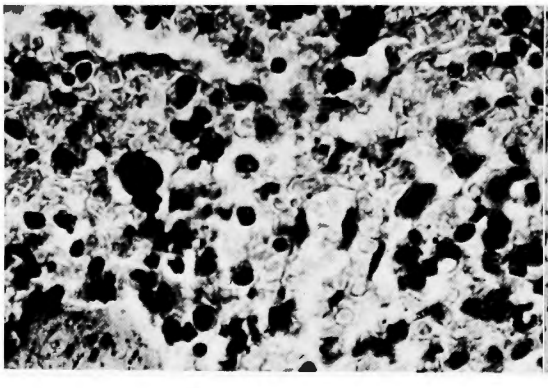

Photo. 9 (×600), Spleen, No. 35 Hemosiderosis in the splenic pulpa is observed.

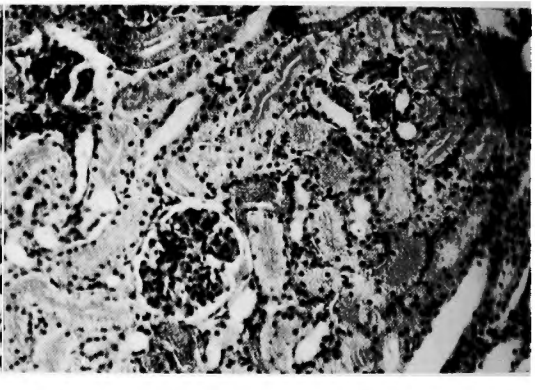

Photo. 10 (×150), Kidney, No. 35 Congestion and tubular degeneration are observed.

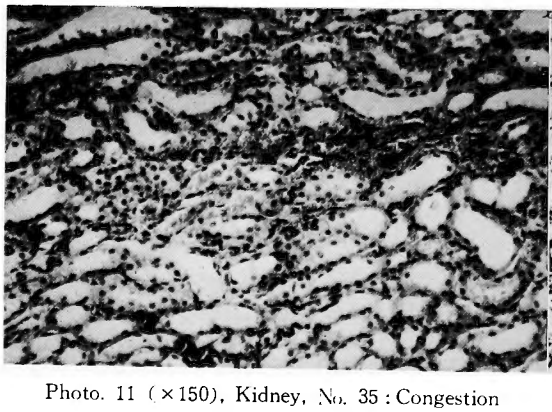

Photo. 11 (×150), Kidney, No. 35 : Congestion accompanying hemorrhage is seen in the border region between cortex and medulla.

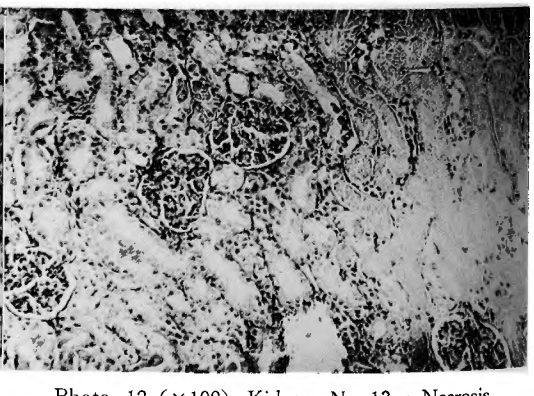

Photo. 12 (×100), Kidney, No. 13 : Necrosis due to infarction is seen. (right side)

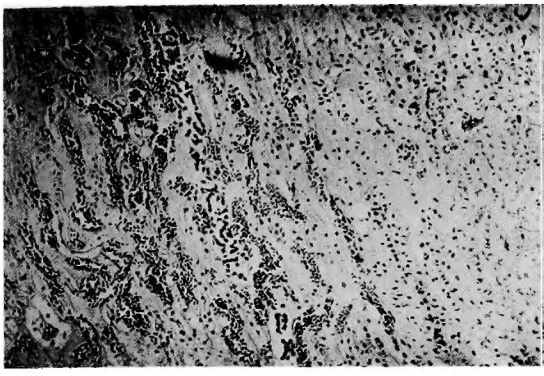

Photo. 13 (×100), Kidney, No. 13 Necrosis due to infarction is also observed in the medullar region. (right side)

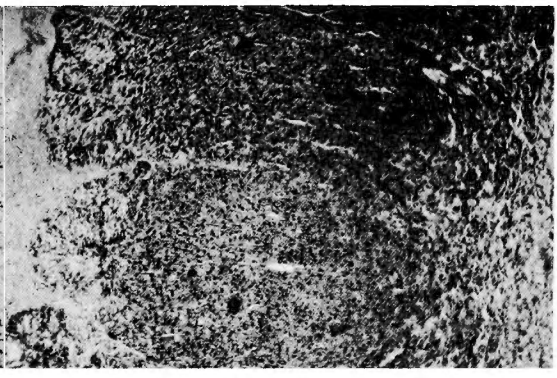

Photo. 14 (×100), Adrenal gland, Normal picture in Sudam III stain.

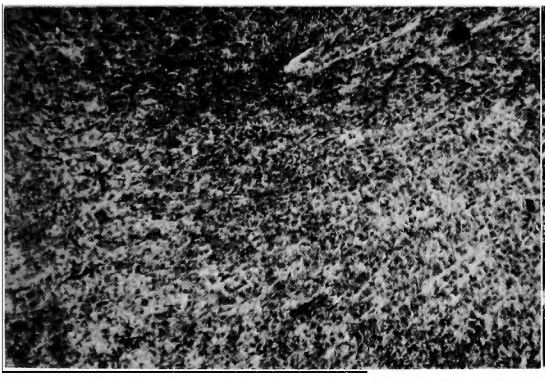

Photo 15 (×100), Adrenal gland, No. 44 : Considerable decrease of lipoid granules is seen.

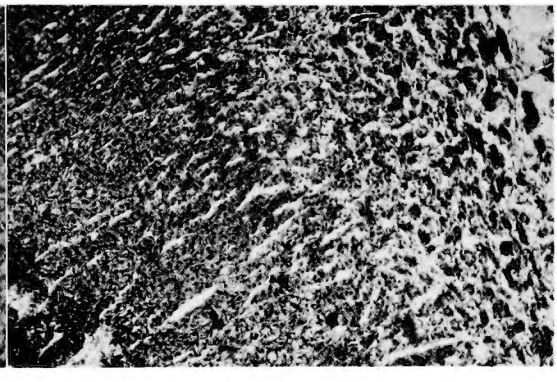

Photo. 16 (×100), Adrenal gland, No. 39 Disarrangement of cell plate is present, but lipoid granules are almost normal.

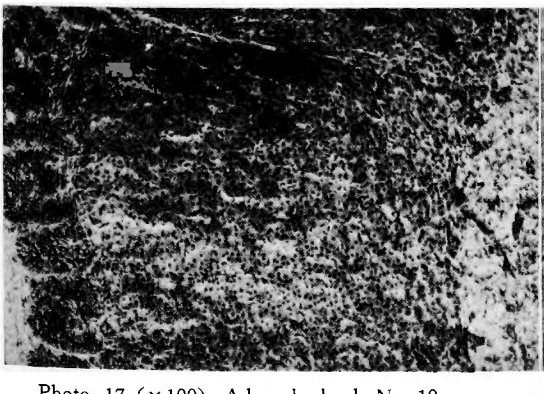

Photo. 17 (×100), Adrenal gland, No. 18 : Histological change is minimal.

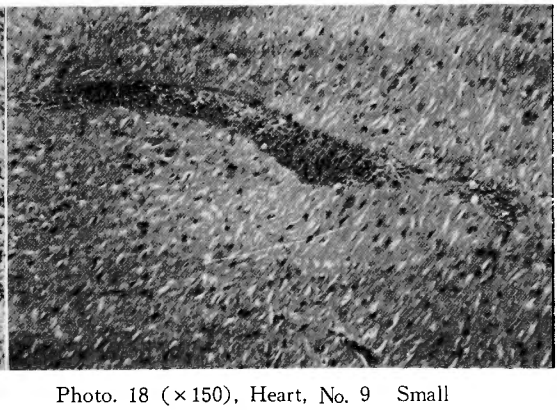

Photo. 18 (×150), Heart, No. 9 Small hemorrhagic lesion is present.

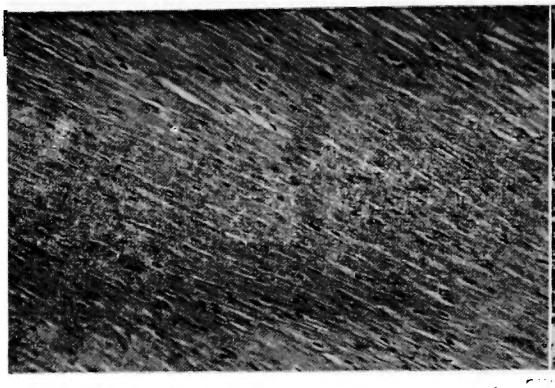

Photo. 19 (×150), Heart, No. 39
No histological change is seen.

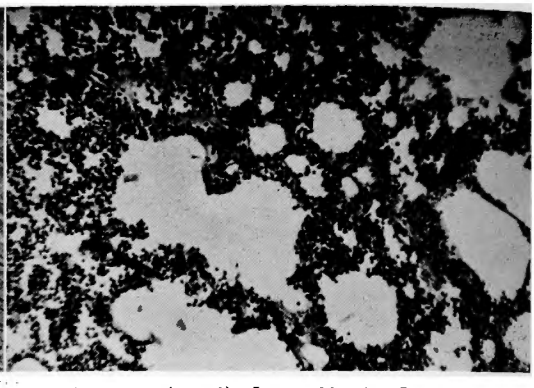

Photo. 20 (×150), Lung, No. 6 : Congestion with hemorrhage is seen.

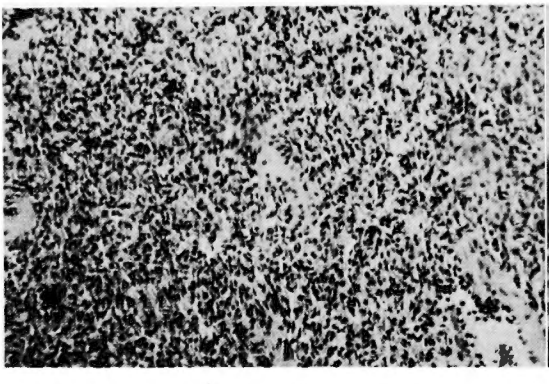

Photo. 21 (×150), Lung, No. 25 : Intraalveolar exudate and atelectasis are seen.

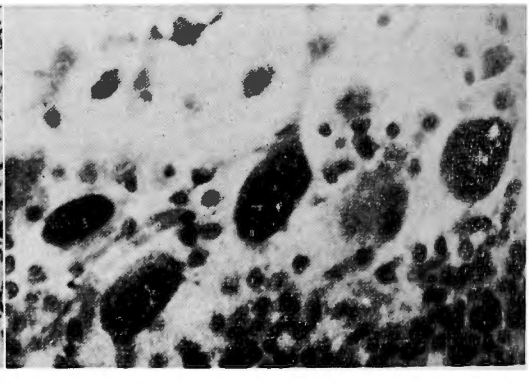

Photo. 22 (×600), PURKINJE cells, No. 42 (NISSL's stain) Swollen PURKINJE cells are present.

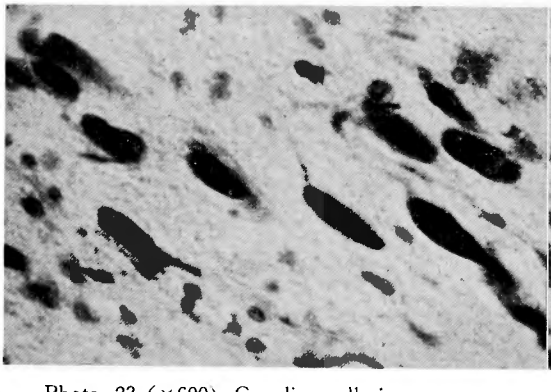

Photo. 23 (×600), Ganglion cells in hippocampus, No. 42 Picnotic ganglion cells are present.

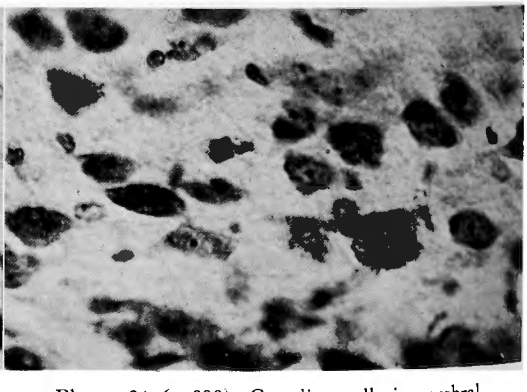

Photo. 24 (×600), Ganglion cells in cerebral cortex, No. 42 :
Mild chromatolysis is seen.