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THE PREVENTION OF INTRAVASCULAR THROMBOSIS BASED UPON ELECTRIC PROPERTIES

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ABSTRACT

Although many theories have been reported as to the cause of venous thrombus, the three factors listed and explained by Virchow are still widely accepted. Various experimental studies have been done based upon the assumption that the disturbance in blood vessel intima was the main causing factor for the formation of thrombus due to mechanical or chemical injuries to the intima.

A report was made by Abramson in 1927 as to the negative charge nature of the surface of blood cells, and later in 1953, Sawyer *et al.*, reported that the intima of blood vessels was negatively charged in the physiological condition, and that thrombus was not caused by the electric repulsion of the blood cells and the intima. Furthermore, it was said that when the intima was mechanically or chemically injured, the negative charge of the intima underwent reversion of polarity, and the thrombus formed.

This investigation was carried out *in vitro* by using autoradiography to determine the necessary intensity of electricity, duration of electrification, and other factors which were required for the formation of thrombus by positive charge under normal condition. At the same time, to test the effect of thrombus prevention, experiments using negative charge were conducted in which the intima was mechanically injured to easily make thrombus. And, after applying electrification, the intimal surface of the blood vessel was observed microscopically. Negative charge was applied to the transplantation of artificial blood vessel for vein. Wrinkled teflon graft about 3 cm in length was transplanted to the inferior vena cava of an adult dog. A platinum electrode was coiled around the part of end to end anastomosis and electrified as a negative electrode. After a certain period of time, patency was examined by venography. Patent cases were all of very early stage of electrification, and no thrombus formation occurred with a case of negative electrode of platinum coil used to prevent kinking and pressing.

INTRODUCTION

Many discussions of research done as to the cause of thrombus have been reported[1]. Even today, the three factors advocated by Virchow: 1) stagnation of blood circulation, 2) disturbance of the vessel wall, and 3) hypercoagulability of blood, are accepted as the cause. However, discussions are divided as to which one of these is the primary cause. For instance, those insisting

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that the stagnation of blood circulation is the major cause also consider the exasperation of blood coagulability as a cause. While others emphasize the anatomical relations of the blood flow as well as the acceleration of the blood platelet adhesiveness. They also have, recently adapted an idea of rheology, studying the relation of blood adhesiveness and shear rate, and also the increase in prothrombin activity and the number of blood corpuscles and platelets. Hashimoto[2] emphasized that an injured intima is the main factor which cause occurrence of thrombus.

Extensive studies have been made in our laboratiry[3] on thrombus formation by causing a mechanical or chemical injury to the intima.

In these studies, studies were made to explain the point *in vivo, in vitro,* histopathologically, and autoradiographically.

With special attention to the various electrical properties existing between the intima and the surface of blood cells as a causative factor in thrombus formation, experiments were conducted for the prevention of formation of the thrombus.

Abramson[4] reported, in 1927, that the surface of blood cells was negatively charged, and, in 1931[5], he observed the galvanotaxis of the red blood corpuscles by electrophoresis. Rosenthal and Tobias[6] mesured in 1948, the time necessary for blood to coagulate, and Henstell[7] confirmed it in 1949. Samuell *et al.*[8], reported on the electrical resistance.

In Japan, Senda[9] referred to the galvanotaxis in his description of the bionomics movement of white cells. In 1953, Sawyer *et al.*[10][11], reported that the intima of blood vessels, in its normal condition, was charged with negative electric load, and under the same condition, no thrombus formation could occur by the electric repulsion between the blood cell surface and the intima, but when mechanical injury was given to the intima, polarity was reversed or lost, and thrombus was formed.

Sawyer *et al.*[12], used the mesentery of a rat to put positive electrode in the blood vessel to create an electric field, and the coagulation of red blood cells was observed microscopically. They[13] also transplanted artificial blood vessel in the vein of an adult dog and measured the electrical potential difference. They noticed in this experiment a relation existed between the electrical potential difference and the thrombus formation.

Fukuta[14][15] reported the measurements of the electrical potential difference caused by the transplantation of artificial blood vessel using a precision potentiometer.

Red blood cells and platelets were labelled by radioactive isotope Cr^{51}, and the amount of positive charge was determined to form the thrombus by using jugular and femoral veins of an adult dog in a normal condition, and some histo-pathological finding were acquired under the same condition. At the same time, mechanical injury was given to the intima to produce a condition

which predisposed to formation of thrombus, and through negative charge, the prevention of thrombus at set times was checked with an autoradiograph and by a histological method. As an applied experiment, an artificial blood vessel was transplanted in the inferior vena cava of an adult dog, and negative direct current was applied to prevent thrombus formation. After transplantation, the occulsion or patency was examined by venography, and the importance of early electrification was noticed.

Experimental Studies on the Prevention of Intravascular Thrombosis Based upon Electric Properties between the Intima and Blood Cells

EXPERIMENTS

I. Experimental study on the galvanotaxis of red blood cells

1. Purpose and method of experiments

The studies conducted by Sawyer *et al.*[16)17)], revealed that potential difference existed between the intima and the externa of blood vessels in their normal conditions, that the intima was electrically negative against the externa and that the potential difference ranged from 1 mV to 5 mV. They also reported in physiological conditions that the external surface of blood cells was charged negatively against the internal part, the intima of blood vessel and the surface of the blood cell were electrically repulsing each other, and that this repulsion prevented thrombus and the attachment of the blood cells to the intima.

The minimum intensity of electric current in the red blood corpuscle indicative of galvanotaxis was measured *in vitro*. The conditions of attraction and repulsion of the red blood corpuscles at both electrodes were microscopically observed. After a period of time, morphological changes of the red blood corpuscle was examined. Experiments were carried out to determine the change of potential difference due to pH variation by the reaction of acid and alkali. Experiments were conducted on the electrical effect of heparin upon the red blood corpuscles at the electrodes.

2. Experimental materials

Dry cell battery	1.5 V
Variable resistor	0–2 kΩ
Ammeter	0–5 mA±0.2 mA
Voltmeter	0–10 V±0.4 V

Platinum needle electrode or silver-silverchloride electrode
Conductor (copper wire)
Electrode-fixing plate
Thoma blood cell count plate and melanjule
Microscope

Microscope photographic apparatus

3. Methode of experiments

Blood was withdrawn to 1 scale by means of melanjule for red blood corpuscles measurement. It was diluted to 101 with saline, and well shaken. As in the method of counting red blood corpuscles, the diluted blood was transferred to the Thoma plate, and both grooves were filled. Platinum wires as electrodes were gently inserted into both grooves and fixed tightly by fixing plate. The electrodes were connected to the conductor, and dry cell battery was connected to the conductor to form a circuit. When the swith was turned on, electric resistance was adjusted with a variable resistor to make the intensity of current free and to read the value at the same time by mean of ammeter and voltmeter (Fig. 1).

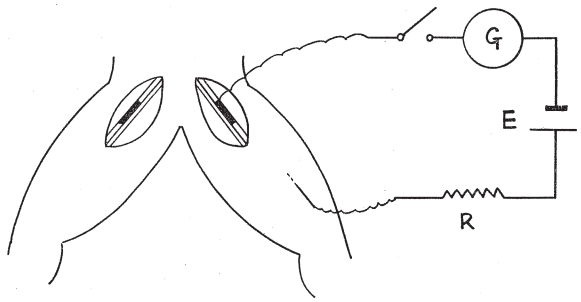

FIG. 1. Schema; Electrification to femoral vein on an adult dog *in vivo*. Negative charge was conducted for the purpose of preventing thrombus formation.

After checking by microscopic observation that the red blood corpuscles were settled on the Thoma, the switch was turned on, and the intensity of electric current sufficient to move the red blood corpuscles was checked with various resistor and recorded. With enough ampere available to cause galvanotaxis, several attempts were made to measure the time required for the movement of the red blood corpuscle one unit on the Thoma. Then, the slow changes occurred at both electrodes and the morphological nature of such changes were also observed. Qualitative check was made by the use of litmus paper of the change of pH which was assumed to be brought about by the electrolysis of saline. Furthermore, potential difference was measured with a high precision direct current potentiometer from which the relative value of pH was calculated. Finally, 10 mg/ml heparin, at a rate of 0.02 cc for 2 ml of saline, was added to saline, and blood was mixed in the same manner as before. The blood sample was electrified and the changes at both electrode and the morphological changes of the red blood corpuscles was observed, and the changes in pH at the end of electrification was also tested (Fig. 2).

4. Result of experiments

(1) Measurement of the minimun current indicating galvanotaxis of the red blood corpuscles

The intensity of the minimun current to cause the reaction of the blood corpuscles was determined by an apparatus in which the current is raised slowly from the minimun by manipulating the voltage and resistance.

The measurement disclosed that galvanotaxis was shown for the first time at 3 V 1 kΩ and 0.52 mA. This made it clear that at least 0.52 mA of current was required *in vitro* to give rise to the galvanotaxis of the red blood corpuscles. In this experiment that the internal resistance of a circuit in the apparatus was measured to be approximately 3.75 kΩ, and that the experiment *in vitro* the degree of internal resistance was influenced by strength of electric current.

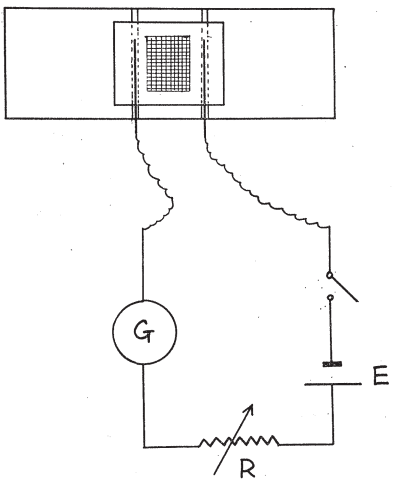

FIG. 2. Schema; Measuremen t of red blood cell galvanotaxis *in vitro*. Red blood corpuscle indicated positive galvanotaxis.

(2) Changes in blood dilution at both electrodes

a) Changes at the positive electrode after electrification

The changes at the positive electrode were examined by the electrification of 3 V, 58 Ω and 0.93 mA. At the positive electrode 2 minutes after electrification, air bubbles were produced at which time the flow of the red blood corpuscles was noticed, and the gradual migration of the red blood corpuscles to the platinum electrode was observed. After 5 minutes, a considerable attraction and gathering was observed.

b) Changes at the negative electrode after electrification

In 2 minutes 30 seconds after electrification under the same condition as before, slightly larger air bubbles at the positive electrode were noticed, and then the current was cut off red blood corpuscles were observed scattering and dispersing. The changes at the negative electrode were within 5 minutes. There were any red blood corpuscles around the negative electrode as there were destruction of the red blood corpuscles rather than dispersion.

(3) Morphological changes of the red blood corpuscles at both electrodes

a) Blood diluted with saline

There was no unusual morphological change at the positive electrode, but at the negative electrode there were some collection, dispersion, and destruction of the red blood corpuscles. With 0.6 mA electrification, the red blood corpuscles did not quite reach the positive electrode, and at the negative

electrode, destruction of red blood corpuscle was observed in 12 minutes. The sequence of reaction was as follows: when electrified at 0.6 mA, the red blood corpuscles moved away from the negative electrode, and in 5 minutes, they became reduced in size and more spherical in shape. This was considered to be due to water absorption. They became brilliant and in the meantime the red blood corpuscles lost the clear-cut contour and fade, and suddenly was destroyed. No movement of the red blood corpuscles was observed during this time.

The morphological changes observed in these experiments confirmed that each electrode became acid and alkali in nature respectively by the electrolysis of saline when electrified with direct current, and that the change in pH was assumed to affect the morphological state of the red blood corpuscles. At the end of electrification, litmus papers were inserted in both grooves for qualitative determination; at the positive electrode, the litmus paper showed acidity, and at the negative electrode it showed alkalinity.

In the following experiment, without electrification, HCl and NaOH of 1/10 N respectively were added to both grooves of Thoma blood cell count plate, and the morphological changes of the red blood corpuscles were observed. At the side to which HCl was added, hardly any change of the red blood corpuscles was observed, while there was destruction at the side which NaOH was added.

b) Blood diluted with heparin added to saline

Heparin is known to be an anti-coagulant, and has the nature of negative electrification. Nova-heparin solution of 10 mg/ml was added to saline diluted blood 0.01 ml to 100 ml of blood. Two 1.5 V dry batteries were connected in series and electrified with resistance of 1 kΩ. The intensity of the electric current was 0.44 mA and the internal resistance of the circuit was 5.36 kΩ. Within 10 minutes after electrification, the red blood corpuscles gradually were destroyed at the negative electrode, and in 14 minutes, all the red blood corpuscles were completely destroyed on the calculation plate.

The negative electrode was formed to be strongly quantitatively alkali at the end of electrification. The positive electrode was found to be neutral.

It was found in this experiment that heparin increased alkalinity, in other words, strengthened the negative load.

(4) Measurement of pH at both electrodes

The difference in pH of diluted blood at the electrodes at the end of the above-mentioned experiment was measured by the differentiometer. The result showed that there was the potential difference of 38 μV.

According to Nernst, the electromotive force E arising between the electrodes is represented by the following formula[18].

$$E = \frac{RT}{F} \ln \frac{[H^+]^1}{[H^+]^2}$$

R=gas constant (8.312 V Coulomb T^{-1})
T=absolute temperature
F=Faraday constant (96.500 Coulomb)
ln=Natural logarithm

Natural logarithm is converted into constant logarithm, and with numerical values put in the above formula.

$$E = 0.0001983\ T \log \frac{[H^+]^1}{[H^+]^2}$$

$$-\log[H^+]^2 = -\log[H^+]^1 + \frac{E}{0.0001983\ T}$$

The logarithm of hydrogen ion concentration with a negative symbol is written in pH as follows.

$$pH_2 = pH_1 + \frac{E}{0.0001983\ T}$$

Based upon the fundamental electrode, the potential difference E is measured. The pH of a solution with the density of H^+ is represented by the following formula.

$$pH = \frac{E}{0.0001983\ T}$$

E is a voltage measured with V unit.

Therefore, the potential difference of 1 mV converted into the difference in pH is 0.018. The change in pH in saline diluted solution after electrification was anticipated to be considerably great at both electrodes by electrolysis, but by actual measurement it was of small potential difference of 38 μV. When the potential difference was measured by the use of plasma, the difference was as small as 24 μV. It was assumed that the positive polarity of the red blood corpuscles depend more upon bioelectric nature than the chemical nature.

5. Consideration

The strength of the minimun current necessary for galvanotaxis of the red blood corpuscles was 0.5 mA *in vitro*.

Judging from the change in pH after electrification, negative charge was influenced by heparin, and the use of it brought a change in galvanotaxis of the red blood corpuscles.

The chemical changes of the acid or the alkali appeared to slighty affect the magnitude of galvanotaxis.

Electrification caused a change in pH by the electrolysis of the electrolyte, but since its value was exceedingly low, the galvanotaxis was assumed to more physical and bioelectric rather than the change in pH itself.

II. Experimental research on the thrombus formation by direct current electricity and its prevention, in particular, on autoradiography

1. Purpose and method of experiment

(1) Purpose of experiment

Although experiments[19)20)21)] have been reported as to the formation and prevention of thrombus by the change in the electric load on the intima of blood vessels and surface of blood corpuscles, no attempt was made to observe the visible change of the blood corpuscles at regular interval during the electric change. As a means to observe quantitatively and visibly the stage of thrombus formation and to record very detailed data on the changes, the labelling by radioactive isotope of the blood corpuscle may be mentioned.

Inoguchi and Yagi[22)] conducted experiments in 1956, concerning the capacity of selective adhesion of radioactive thrombocyte on the disturbed wall of blood vessel and proved by the use of a Geiger-counter that the adhesive coefficiency of the disturbed wall of blood vessel was greater than that of the normal one, and they found this tendency was more marked in the blood platelets than in the red blood corpuscles.

In 1959, Shimizu[23)] conducted experiments on morphology using the autoradiograph. Autoradiography is a technique directly record the distribution of radioactive isotopes on the emulsion by the use of the sensitizing capacity of the radioactive rays[24)]. This obtained picture is called an autoradiograph. By this method, the radioactive isotopes can be located according to the blackened spot of the enmulsion, quantitative change is determined by the degree of blackening of the autoradiograph.

The degree of blackening by changing in the amount of electricity, the intensity and time was done to attempt to prevent the thrombus formation by negatively charging the injured wall of blood vessel, and the change in blackening was examined. The effect of positive charge when heparin, nialamide and dextran were mixed, was also investigated, and it was proved that there was no difference in the effect of injury and electrification upon the formation of the thrombus.

(2) Experimental materials

Adult dogs were selected as experimental animals, and the jugular and femoral veins on both sides, and often the inferior vena cava were used. After double ligature, blood vessels were dissected. The blood of the dog was previously collected, and the red blood corpuscles and the blood platelets were separated and labelled.

The radioactive isotope used in the experiment were P^{32} and Cr^{51}. X-ray film, process plate, and autoradiographic plate are available for sensitizing purpose. Of these, the autoradiographic plate has the finest grain giving the sharpest sensitivity and the best resolving power. Of the two kinds of autoradiographic plate, contact type and stripping type, contact type is suitable

for contact method, and stripping type for stripping method. For this experiment, Fuji-films were used. For development, Corectol was used and for fixing Fujifix was used.

Fuji Autoradigraphic Plate (for Contact)
EM-type ET-2 E 15 microns
Fiji Autoradigraphic Plate (for Stripping)
EM-type ET-2 E 15 microns

(3) Method of experiments

a) Preparation of the blood vessel fragments expanded

The jugular and femoral veins of an adult dog were dissected to a length of 2 to 5 cm after double ligature. Radioactive isotope labelling were done *in vivo* and *in vitro*. *In vivo* the majority of radioactive isotopes enter in the external cavity of blood vessel and excreted in the urine. Therefore, the method of *in vitro* was employed for this experiment. The dissected blood vessel with both ends tied were filled up with blood. For labelling and easier observation of the blood vessel intima, the blood vessel cavity was cut open vertically after electrification and extended using thread. The dissected vessel was expanded and fixed on a slide glass (Fig. 3).

b) Label to the red blood corpuscles[25]

Prior to excision of the jugular and femoral veins, 10 ml of blood was taken from the femoral vein and 100 μC of Cr^{51} was added to it, and immediately kept in 37.0°C. Every 30 minutes for two hours the specimen was mixed. After two hours, it was centrifuged at 1,500 rpm. Then, the upper plasma was thrown away and the sediment was washed with an equal amount of saline. This was mixed and centrifuged again for three times, and finally saline was used to delute up to 10 ml. This way the labelled red blood corpuscles was obtained.

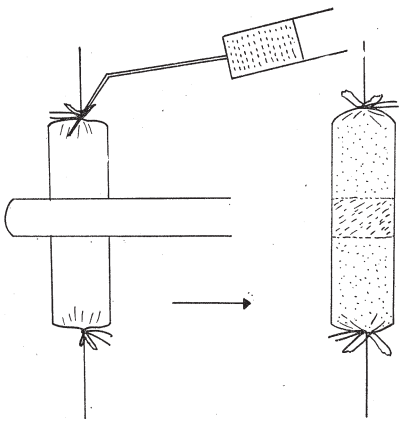

FIG. 3. Schema; Clamp to the blood vessel *in vitro*, and label to blood cell corpuscles by radioactive element.

c) Label to blood platelets

First of all, blood platelets were separated. It was necessary that the blood platelets should be intact with a large collectivity and high degree of purity. But it was extremely difficult to have all these conditions satisfied. The method of separation by Morita *et al.*, was used. Silicon was used for coating on the glass surface to make the inside of container non-wettable. For this purpose, Shin-etsu Kagaku's silicon oil KT 99 was used. The temperature was 200°C,

and the baking time was 15 minutes. As the best-known anticoagulator, the highest quality for the preservation of the blood platelets, di-sodium ethylen di-amino tetra acetate (Na_2 EDTA) Sequesterene Na_2 was used. The 0.5 ml solution of 10% Sequesterene Na_2 in 5% glucose solution was added to 10 ml of collected blood solution. It was immediately cooled to 4°C. Next, it was centrifuged at 1,000 rpm for 5 minutes, so blood plasma containing a large number of blood platelets was obtained. The suspension on the top was poured into other container. In this process, a little quantity of blood was mixed so resuspension was done. The blood platelets were centrifuged at 2,500 rpm for 30 minutes for. The sediment of platelets was resuspended in the blood plasma. Thus plasma suspension contained only of blood platelets. This procedure was done under low temperature.

Secondly, in regard to the labelling to the blood platelets, 10 ml of blood was collected from a dog, and the blood platelets were separated as previously. It was resuspended in the blood plasma almost equivalent to the amount of blood collected. 5 ml of this suspension was taken, and 50 μC of Cr^{51} was added. After the mixture was left standing for two hours at 37°C, it was centrifuged at 2,500 rpm for 30 minutes This supernatant fluid which contained excess radioactive isotope was discarded. The plasma was resuspended repeatedly until no excess radioactivity was traced in the supernatant fluid. Since Shimizu[23] used the Geiger counter to check, we washed the sediment three times following his procedure.

d) Preparation of autoradiograph of the blood vessel wall

The vein dissected after double ligature was put in saline. The internal

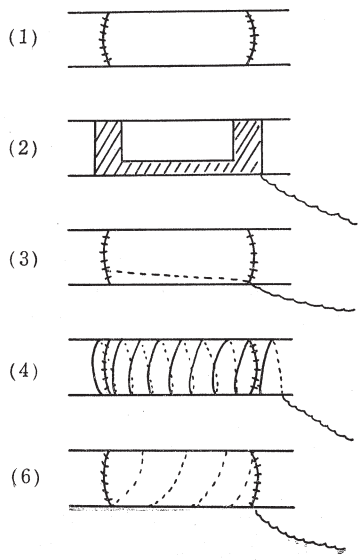

FIG. 4. Schema; Electrodes after artificial blood vessel transplantation.
(1) Control; Artificial blood vessel transplantation (Teflon-grafting).
(2) Platinum plate electrode.
(3) Electrode in which golden wire was woven.
(4) Platinum wire electrode in a coil condition.
(5) Platinum wire electrode as a suture.

cavity was washed slightly with saline to completely wash away the blood. Blood or plasma containing radioactive isotope, almost equal to the amount of blood in the cavity, was introduced one end.

For positive charge, an electrode made with platinum plate was coild over the entire length of the blood vessel to make it the positive electrode. On the other hand, to make a field, an opposite electrode was put in saline to make it the negative electrode. 3 V dry cell batteries were used and 6 stages of electrification each with 5 minutes intervals from 5 to 30 minutes were performed. The internal cavity was opened after 30 minutes, including the time of electrification, and blood vessel was extended. As control, platinum electrode was coild but not charged; then, after 30 minutes, it was removed and the blood vessel was extended. Then 6 V was applied at 6 stages from 5 to 30 minutes. During the time of electrification, the ammeter continuously measured the strength of current and recorded the mean value.

Negative change experiments were also conducted as an attempt to prevent thrombus formation. Clamp for one minute was used to give a mechanical injury to the intima, and blood or blood plasma containing radioactive isotope was injected immediately. Platinum electrode was coild and left 30 minutes on the control. For the test, platinum electrode was coild, and at 6 stages of 3 V from 5 to 30 minutes, and 6 stages at 6 V were given the negativecharge. At the end of 30 minutes, the blood vessel was opened, and the internal cavity was observed.

Macroautoradiography (contact method).

At the end of electrification, the internal cavity of the blood vessel was opened and carefully washed with saline. The opened vein was put on the slide glass and was bound with suture at four edges for extension. Then, it was immersed twice in 1% ether alcohol and left in it for one night. On the next day, it was immersed in saline. One drop of celloidin was dropped to form a thin film, and it was gently raised from the lower part to cover the surface with celloidin film. Moisture was wiped away completely, and the specimen was put in a refrigerator one day and dried at low temperature. In a dark room, the intima surface was lightly joined and exposed the specimen for seven days and kept in a refrigerator at 1°C to 4°C. Silica-gel was used as a drying agent, Corectol as the developer, and Fujifix as the fixer.

Microautoradiography (stripping method).

Similar to the contact method, the extended fragment of blood vessel was covered with celloidin film. In a dark room, stripping plate was immersed for one minute in glycerin, then water warmed up to 20°C was added. From a part of the plate which was scratched with a knife previously, it could easily be peeled off. The extended dissected specimen was put in saline, and the surface of emulsion of the film peeled off and the specimen were raised with caution not to have air bubbles. Water drips were wiped off with a

filter paper and the specimen dried. After this, it was exposed in a refrigerator for two weeks similarly as in contact method, and developing and fixing were done.

2. Result of experiments

(1) Positive electrification

Following the described method of positive electrification experiment, the specimen was prepared for autoradiography and left 30 minutes simply with the electrode coiled around. Autoradiographs with the time of electrification at 3 V and 6 V from 5 to 30 minutes was conducted according to contact and stripping method, with the labelled red blood corpuscle and the blood platelet. The autoradiograph was put in an ordinary photograph side by side with the extended specimen by contact photography.

a) Control (Fig. 5, Fig. 6)

In the control, only with the electrode coild and no electrification, autoradiography was done 30 minutes later and no positive findings were found in either of the red blood corpuscle or the blood platelet.

b) Positive electrification (red blood corpuscle label)

In the case of 3 V, the intensity of the current flowing in the field was 3.8 mA to 4.0 mA. No positive finding was found after 10 minutes of electrification by contact method, but after 15 minutes, blackening did appear and also in the case of 30 minutes. In the stripping method, within 10 minutes, a slight blackening was faintly noticed, and also in the 15 to 30 minutes cases. With the 30 minutes electrification, comparatively heavy blackening was obtained. To compare contact and stripping method, the latter seemed to show mere sensitive reaction. With 6 V electrification, both began to show blackening in 10 minutes, and these blackening became quite definite after 30 minutes

TABLE 1. 3 V Positive Electrification (red blood corpuscle label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	3/0	3/3.8	3/3.8	3/3.9	3/3.8	3/4.0	3/3.8
Cont. meth.	−	−	−	+	+	+	+
Strip. meth.	−	−	±	+	+	+	++

TABLE 2. 6 V Positive Electrification (red blood corpuscle label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	6/0	6/5.9	6/5.9	6/5.7	6/5.7	6/6.0	6/5.9
Cont. meth.	−	−	+	+	+	+	++
Strip. meth.	−	−	+	+	+	+	++

TABLE 3. 3 V Positive Electrification (blood platelet label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	3/0	3/3.9	3/4.0	3/3.9	3/3.8	3/4.0	3/4.0
Cont. meth.	−	±	+	+	+	+	++
Strip. meth.	−	±	+	+	+	+	++

TABLE 4. 6 V Positive Electrification (blood platelet label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	6/0	6/6.2	6/5.9	6/5.9	6/6.1	6/6.0	6/6.0
Cont. meth.	−	±	+	+	+	+	++
Strip. meth.	−	+	+	+	+	++	++

electrification, and the change in the voltage itself was beleaved to be more markedly than 3 V.

c) Positive electrification (blood platelet label)

A method similar to the positive electrification for the red blood corpuscles was employed. With 3 V, 5 minutes charged cases in both methods, a faint appearance of blackening was observed, but in 10 to 25 minutes electrification, definite blackening was obtained. Also with 6 V, electric current from 5.9 mA to 6.2 mA was obtained. But with 5 minutes electrification, in the contact method, a slight but definite blackening was noticed. In 25 and 30 minutes electrifications, the areas became markedly distinct (Fig. 7).

In comparing the time of appearance of blackening with positive charge in regard to the red blood corpuscle label and the blood platelet label, there was difference of 5 minutes. In other words, the labelled blood platelet produced blackening one step quicker at the time than the other.

The relation of these two labelled cells in the system of thrombus formation may be stated briefly as follows. Judging from the formation system of white thrombus and red thrombus, it seemed natural that the difference is due to the preceding adhesion of blood platelets to the intima, which is described in the paragraph of consideration. The following was an outlined description of the result of experiment at the time of positive charge.

(2) Negative electrification after clamp

Shimizu[23] stated that the adhering of blood platelets to the intima endothel cells is a result of injury to the intima and referred to the formation of white thrombus and red thrombus. The intima was injured to greate a condition that the thrombus would easily form, and then negative charge was applied and put in the autoradiograph.

a) Control

As a control, the intima was injured by the application of clamp for one minute on the previously prepared blood vessel, and a separate application of clamp on the tissue was applied. This was extended to 30 minutes, and an increase of blackening was noted.

b) Negative electrification (red blood corpuscle label)

Clamp was applied similarily to a control to cause mechanically injury. Immediately following, electrification with 3 V, and 6 V were conducted at each stage from 5 to 30 minutes. The adhesion of the red blood corpuscle to the intima was examined by autoradiography. After 5 minutes, blackening was still observed and also 10 minutes later. In the case of 15 minutes later, blackening disappeared, and in each case to 30 minutes later, it was not observed. Same result were obtained by both contact and stripping method. In every electrification, at 6 V, after 15 minutes, blackening was found to have disappeared. Similarily at 6 V with positive charge, 30 minutes electrification showed a distinct change in the tissue macroscopically. It has been observed that the time of electrification had larger effect upon the prevention of thrombus formation than the strength of electric current. This result are shown the table (Table 5) (Fig. 8).

TABLE 5. 3 V Negative Electrification (red blood corpuscle label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	3/0	3/3.8	3/3.9	3/3.9	3/3.8	3/4.0	3/4.1
Cont. meth.	++	+	+	−	+	−	−
Strip. meth.	++	+	+	−	−	−	−

TABLE 6. 6 V Negative Electrification (red blood corpuscle label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	6/0	6/6.0	6/6.0	6/5.9	6/6.2	6/6.2	6/6.2
Cont. meth.	++	+	+	−	−	−	−
Strip. meth.	++	+	±	−	−	−	−

TABLE 7. 3 V Negative Electrification (blood platelet label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	3/0	3/3.8	3/3.9	3/3.8	3/4.1	3/4.0	3/4.0
Cont. meth.	++	+	+	−	−	−	−
Strip. meth.	++	+	−	−	−	−	−

TABLE 8. 6 V Negative Electrification (blood platelet label)

Time	cont.	5′	10′	15′	20′	25′	30′
V/mA	6/0	6/5.9	6/6.1	6/6.2	6/6.1	6/6.0	6/6.1
Cont. meth.	++	±	−	−	−	−	−
Strip. meth.	++	+	−	−	−	−	−

b) Negative electrification (blood platelet label)

Similar to the red blood corpuscle label, autoradiograph was made with the blood platelet label. As a means of control, blood vessel was clamped in the same manner. Observations were made at 3 V and 6 V of electrification, and at each stage from 5 to 30 minutes according to contact and stripping methods.

Distinct blackening was noticed in a control similar to the red blood corpuscle label. With 3 V electrification at 5 minutes, blackening still remained, but at 10 minutes electrification, it disappeared by stripping method. In 15 minutes electrification by contact method, the disappearance of blackening was definite. Then, up to 30 minutes electrification, no blackening was observed. In case of 6 V electrification, from 10 to 30 minutes, blackening disappeared. Comparing the red blood corpuscle label and the blood platelet label, the former required more than 10 minutes for the disappearance of blackening and the latter began to disappear within 5 minutes of electrification. It was definite that the response of the blood platelets upon negative charge, similar to positive one, reacted quicker than the red blood corpuscles.

(3) Relation of direct current electricity with chemicals in the blood platelet label

Experiments have been reported in which the intima was mechanically injured, thus a condition for thrombus formation was created, and after anticoagulant was given, there is no confirmation of thrombus formation. Instead of mechanically injuring the intima, positive charge was applied by autoradiography to determine whether thrombus would be formed. Primary attention was given to heparin. 10 mg/kg heparin was intravenously injected and after 30 minutes, blood was collected and the blood platelet was labelled. Then, heparinized blood plasma was added, and autoradiographed. As the results of this experiment by contact method, blackening was not observed at all. It became clear that heparin acted as a preventing agent of thrombus formation even in the case of applying positive charge.

It has been experimentally proved that nialamide prevent white thrombus formation, namely blood platelet thrombus. Histological examinations were done and reported[27]. Thrombus formation was checked in the early stage with the blood platelet and the red blood corpuscle label in the case of 3 V and 30

minutes electrification. The experiments by the intravenous injection of 50 mg/kg nialamide showed no blackening. Finally, research was done to determine the relation of blood coagulation in adhesiveness from the standpoint of rheology[28]. After intravenous injection of 50 ml/kg of dextran, blood was collected and labelled, and it was autoradiographed after the electrification of 3 V and 30 minutes. In the case of the blood platelet label, a slight degree of blackening was acknowledged. In the case of the red blood corpuscle label, it was not conducted because the chemical in the blood would be washed away during the process.

TABLE 9. Positive charge with chemicals (blood platelet label)

Chemicals	heparin 10 mg/kg	nialamide 50 mg/kg	dextran 50 ml/kg
Time	30′	30′	30′
V/mA	3/4.1	3/4.0	3/4.1
Cont. meth.	–	–	–
Strip. meth.	–	–	–

(4) Negative charge in 30 minutes after clamp to the blood vessel intima

As explained before in each case of autoradiography in 30 minutes after clamp, blackening was noted. In other words, it was assured that there was an attachment of blood platelet and of red blood corpuscles to the intima. Although heparin is a strong drug to prevent thrombus formation, when thrombus once is formed, it can not dissolve it. To test whether negative charge would act as heparin does or whether it could separate the once attached thrombus to the intima and pre ent thrombus formation, 30 minutes electrification at 6 V was applied observed by means of autoradiograph. According to this experiment, blackening was found both in the labelled blood platelet and the red blood corpuscle. Briefly, negative charge acts similarily to heparin in preventing thrombus formation, but no power to dissolve thrombus after it is once formed. Negative charge should be used in very early stage. Once thrombus formation should occur, electrification application had no effect at all.

3. Consideration

A few considerations were made as a result of autoradiography. In the case of positive charge, both at 3 V and 6 V, the labelled blood platelet showed the appearance of blackening in 5 minutes, while the labelled red blood corpuscle showed blackening at 3 V in 15 minutes, and at 6 V in 10 minutes. Degree of blackening and coulomb seemed to have a certain kind of relationship[29)30)31)], but the time of electrification appeared to be more important factor than the amount of electric current[32)33)].

TABLE 10. Results of Artificial Blood Vessel Transplantation

No.	Weight (kg)	Electrode	Volt	Amp.	Hours	Result
1	7.0	Ag	6	5.5	3	occlusion
2	10.0	Ag	3	5.0	48	occlusion
3	6.0	Ag	6	5.5	24	occlusion
4*	11.0	Ag	6	4.2	4	occlusion
5	15.0	Ag	9	5.0	2.5	occlusion
6	10.0	Pt	6	5.2	6	occlusion
7	9.0	Pt	9	6.0	4	occlusion
8	13.0	Pt	12	7.0	3	occlusion
9*	8.5	Pt	12	6.0	1.5	patency
10*	8.0	Pt	6	8.0	2	patency
11*	9.0	Pt	6	5.8	2	patency
12*	9.5	Pt	6	6.2	2	patency

* No. 4. The case of using artificial blood vessel teflon in which golden wire was woven (Fig. 4 (3)).

No. 9 and No. 10. The cases electrified while making anastomosis or immediately after anastomosis.

No. 11. The case of electrode that platinum wire in a coil condition to be made (Fig. 4 (4)).

No. 12. The case of electrode that very fine platinum wire was applied as a suture (Fig. 4 (5)).

In the case of negative charge of the labelled blood platelet, blackening was not observed after 10 minutes electrification. After 15 minutes, blackening disappeared. Almost the same result was obtained in the case of the labelled red blood corpuscle. In almost all cases of 10 minutes electrification, blackening was observed. In the case of negative charge, the time of electrification seemed to be a stronger factor than the amount of current. As a matter of fact, more effective experiments could be conducted with the minimum ampere and the longer duration of electrification as far as the tissue effect was concerned.

In the case of negative charge it is emphasized that once thrombus forms, it never can be made to disappear, therefore, direct current should by all means be used in the stage of growing thrombus formation. Thrombus formed by the effect of chemicals, by intimal injury such as the clamp, or by positive electrification were not especially heterogeneous to one another. Being different from intimal injury, affect on the blood vessel intima was minimal in the case of positive electrification. This histological finding was described in the next chapter where histlogical views *in vitro* are reported.

III. The changes of blood vessel wall in the case of electrification especially histological views in vitro

1) Purpose and method of experiments

The degree of blackenin in the course of time interval was investigated by autoradiography. Experiments were conducted to check the change in vein wall under the same conditions as above, and blood vessel specimens were

prepared for use as control. Although experiments were *in vitro*, microscopic observation was required to confirm a change in the blood vessel wall by clamp and the effect of direct current electrification upon the tissue injury.

(2) Method of experiments

Jugular and femoral veins of an adult dog were used. These were dissected by double ligature under general anesthesia without injection of radioactive isotope or other drug into blood.

Positive electrification—The dissected blood vessels were coild with platinum plate electrodes, and these were positive side, while the opposite electrode, negative side was put in saline to form a fieled. Control was left for 30 minutes with no electrification. Then it was taken out and both tied ends were cut off, and the suspended blood was gently washed away. Same operation was repeated from 5 to 30 minutes each with positive charge. The intensity of electric current, then, was read at 3 V by an ammeter. At the end of the time of electrification, switch was cut off, and the specimen was left the remaining time to make 30 minutes including the time of electrification. After that, both ends were cut off and the blood was washed away gently. The specimen was fixed in formalin solution.

Negative electrification after clamp—This was done almost identical as in the case of positive charge. After one minute of clamp, platinum plate was coiled round the blood vessel to be the negative electrode, and the other end was made the positive one, and put in saline. Control was only coild with platinum plate, and not electrified. After 30 minutes, it was taken cut and fixed in formalin. Electrification cases were negatively charged immediately after clamp in each case for 5 to 30 minutes. The specimen was left as it was, and at the end of 30 minutes from the begining of electrification, it was taken out and both ends were cut off. Internal blood was gently washed away, and fixed in formalin.

2. Result of experiments

Positive electrification—In the case of positive charge, no change was noticed at 5 or 10 minutes of electrification. But after 15 minutes, a coagulation of the red blood cells at one spot of vein wall was observed, but no large cell infiltration was observed. In the case of 20 minutes electrification, a considerable collection of red blood corpuscles was noted. In the case of 25 minutes electrification, the same change became more conspicuous (Fig. 11, 12, 13). There was no change in the control.

Negative electrification after clamp—Clamp were used to apply pressure for a certain period with a degree of force, prior to negative electrification. In the control, only clamp was applied, and not electrified, injury of tissue was proven and the attachment of blood corpuscles on the intima was observed (Fig. 9, 10). This might be considered as a step in the course of thronbus

formation by intimal injury. In the case of negative charge, even with the 5 minutes electrification, no attachment of blood cells on the surface of the intima was observed. In the 10 and 30 minutes electrification, no thrombus formation was recognized. In every case, it was observed that the partial injury to the blood vessel tissue was included on the surface of the intima as a result of clamp (Fig. 14, 15, 16).

3. Consideration

As described in the result of experiment, it was affirmed that blood cells coagulated by the application of direct current to the blood coagulation cause thrombus to form. Progressive coagulation in the course of time was almost in direct proportion with the degree of blackening in an autoradiograph. The indistinct change on the tissue caused by direct current agreed with the research report of Sawyer *et al.*[43]. It was checked in the case of negative charge that coagulation of blood cells was found in the control, but no blood cell attached on the surface of the intima in 5 minutes after electrification, and in all case of 10 to 30 minutes, it prevents thrombus formation. The intima tissue change by clamp was also definite. Much accomplishment has been made by the experiments to use direct current for the prevention of thrombus formation. The changes in a very early stage after electrification were recognized histologically in this experiment, and they were in agreement with the result of autoradiography.

IV. Application of direct current electricity in the venous transplantation, and the experimental research concerning the prevention of thrombus formation by artificial blood vessel transplantation in particular

1. Purpose of experiments

Based upon the results of these fundamental experiments *in vitro*, it was necessary to observe the change in the vein *in vivo* after electrification and applying the same condition to the artificial blood vessel transplantation of the inferior vena cava. In case there was some difference between the use or not of direct current, the utilization of anticoagulant would be effective in the transplantation of artificial blood vessel in veins. From this point of view of not using an anticoagulant, the experiments were proceeded using the negative charge and not giving attention to the rate of patency.

2. Materials of experiments

Dry cell battery was used, and for electrode, platinum plate or platinum wire was used for the section of anastomosis. For the positive electrode on the other side, platinum needle was used.

Eor the artificial blood vessel, Nakao Filter laboratory' teflon graft was used. Copper wire was used for conductor, and it was covered with vinyl tube so that it would not directly touch the tissue. As additional accessories, the ammeter and voltmeter were used.

3. Method of experiment

An adult dog was used for experiment. Under general anesthesia with barbiturate 0.03 g/ml, hypogastric median incision was made. The skin was opened extraperitoneally and inferior vena cava was exposed approximately 5 cm. Clamps were put on the both ends to stop the blood flow, and it was cut in the middle. Immediately after cutting it was subjected to end to end anastomosis with teflon graft by hand sewing. The length of the vein 3.0 cm. A platinum plate was applied to the part of anastomosis and fixed by coiling it as if it were covering it. This was the negative electrode. The positive electrode was inserted in a nearly muscular tissue. Electrification started with a certain voltage, and the intensity of electric current, then, was read by an ammeter. For voltage, 3 V batteries were put in series, and used at 4 stages from 3 V to 12 V. Conductor was placed in a silicon tube of 5 mm diameter so that it would not touch the tissue directly. When the electrode was cut off resulting in the suspension of electrification, the tube was passed through the neck to the subctaneous tissue of the back, and attached to the ontside of the body so that no exercise might affect it. At the end or in the midst of the anastomosis, electrification was stopped and 20 ml of 60% Urografin was injected into subctaneous vein of the thigh. Venography was done to examine the patent and occlusive conditions. In the case of occulsion, the artificial blood vessel including the section of anastomosis and the joining venous tissue were extracted, and internal cavity was incised vertically for macroscopic observation. In addition, a part of the section of anastomosis was fixed in 10% formalin and subjected to H. E. dyeing to make a histological specimen, and microscopical observation was made.

4. Result of experiment

(1) Control (Fig. 17)

For control, a group of cases was selected which after transplantation of teflon graft, platinum plate electrode was coiled around but no electrification was applied. In all cases, occlusion was acknowledged by the venography in two hours after anastmosis. In these cases of occlusion, the formation of thrombus was distinctively observed in the internal cavity, which was, after venography, removed and vertically incised.

(2) Negative electrification

In all cases of negative charge, occlusion were observed when electrification was applied after some time after blood flow was stopped, and anastomosis was completed. Macroscopically observation of thrombus formation was observed (Fig. 18). But perfect occlusion was not found, that, the status of thrombus was soft and attachment to the blood vessel wall was not observed. Two patent cases recorded by venography, electrification was started immediately after anastomosis, and clamp was stopped in the midst of electrification to resume the blood flow (Fig. 19). In the experiment using teflon graft in

which golden wire was woven and made the conductor which was electrified causing occlusion to occurr. Taking into consideration the fact that effect of the prevention of thrombus formation by negative charge was disturbed by operation with the external causes such as kinking and pressing of graft, platinum wire was coild around the negative electrode to prevent kinking, and negative electricity was charged to the part of anastomosis. In control where no electrification was done, occlusion was recognized (Fig. 20), but in the case of negative charge, it was patent and recorded by the venography two hours later. It was considered of some importance that not only the part of anastomosis, but also the entire wall of artificial blood vessel should be negatively electrified, and the factors such as kinking and pressing should be eliminated (Fig. 21).

5. Consideration

As a mean of venous transplantation, teflon graft was transplanted in the inferior vena cava and negative charge was applied for the purpose of preventing thrombus formation. No anti-coagulant was used in this experiment.

A group of control where no electrification was done, all showed occlusion by venography within two hours.

By mean of average 5 mA negative electrification, the prevention of thrombus formation at the point of anastomosis. All cases electrified a little while after anastomosis was done showed occlusion. A few cases of patency were those electrified in the midst of anastomosis or immediately after it. And when electrification was suspended, thrombus formation occured. Artificial blood vessel coild with platinum wire to be made as an electrode was important as it could electrically prevent thrombus formation and cause no mechanical injury. If anticoagulant were used in this experiment, the result may be better.

DISCUSSION

A series of experiments was conducted in an attempt to make use of the electrical characteristics of blood corpuscles and intima, and to prevent thrombus formation electrically. These are still in the stage of experimentation, but small arteries and veins with low venous pressure easily produce thrombus at the point of anastomosis and bring about occlusion. As a means of solving these problems, experiments based upon electric properties may be applicable in the venous transplantation of artificial blood vessel and in the transplantation of artificial heart valve.

Sawyer *et al.*[35)36)37)] already reported on the blood vessel transplantation of negative charged teflon in order to keep artificial blood vessel charged with negative electric load for a long time. It is desirable as a project in future that if battery be made of smaller size, more easily charged and implanted like the "pace maker", medical treatment to prevent thrombus formation in vein by electrification from early stage over a long period or inter mittently

applied.

For the purpose of the experiments, the use of anticoagilant was completely elimlnated, but the use of it might have given better result. It is certain that if a certain amount of thrombus can be formed quantitatively under a certain condition without affecting the tissue electrically, it will be of great use to the advancement of chemical treatment for thrombus and also for the investigation on the thrombus formation.

CONCLUSION

The red blood corpuscles were suspended in saline to form a electric field and its galvanotaxis was examined, and it showed "positive galvanotaxis". The use of heparin caused a change of galvanotaxis.

In the experiments of positive charge at 3 V and 6 V by autoradiography, blackening was recognized in 5 minutes of electrification of labelled blood platelet. The blood platelets preceeded the blood corpuscles in the attachment of blood cells upon the injured intima; this was the same under positive charge electrification. As an electric factor to form thrombus, the duration of electrification seemed to be more effective than the intensity of electric current. With electrification voltage larger than 6 V, a change in the tissue of blood vessel intima, though insignificant as it might be, was observed. At 3 V, hardly any change was recognized.

By the autoradiography in the case of negative charge, blackening was not observed in the labelled blood platelet after 10 minutes electrification, and in the red blood corpuscle label of 15 minutes. But in 10 minutes electrification, it was recognized. This showed that blood platelet was acting in the first stage both in the occurrence of thrombus formation by positive charge as well as the prevention of thrombus formation by negative one.

Once formed thrombus could never be desolved by negative charge. The function of negative direct current was as useless as heparin after thrombus formation.

Thrombus was caused by positive charge, and it did not differed from the thrombus brought about by mechanical injury of the intima. And when compared with the later, the change in the tissue of the intima was slight.

The histological change in the blood vessel wall, after electrified *in vitro*, almost agreed with the findings of the autoradiograph.

In regard to the application of artificial blood vessel transplantation, good result was not accomplished by the electrification of approximately 5 mA. To prove in the fundamental experiment, electrification should start in a very early stage. In fact, some cases of electrification were patent immediately after anastomosis. It was sure that the most ideal method of the prevention of thrombus formation by negative charge was a long term electrification in the earlist possible stage with the most effective and the most minimum current.

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EXPLANATION OF FIGURES

FIG. 5. Autoradiograph; Control in which injury was conducted to the blood vessel intima.

FIG. 6. Autoradiograph; Control in which injury was conducted to the blood vessel intima. The grades of blackening of intimal injury areas were recognized.

FIG. 7. Autoradiograph; Stripping method. 3 V, 30 minutes positive charge (red blood corpuscle label). Blackening was recognized as well as control clamping case Fig. 6.

FIG. 8. Autoradiograph; Stripping method. 6 V, 20 minutes negative charge (red blood corpuscle label). No blackening was recognized at the clamped intima after negative charge.

FIG. 9. Control; Microscopic finding after clamp to the blood vessel wall. ×100 H.E.

FIG. 10. Control; ×400 Weigert' selastic fibre stain.

FIG. 11. Microscopic finding after 3 V 15 minutes positive charge. ×100 H.E. Thrombus formation began to occur in contact with a positive electrode.

FIG. 12. Microscopic finding after 3 V 20 minutes positive charge. ×100 H.E.

FIG. 13. Microscopic finding after 3 V 25 minutes positive charge. ×100. Weigert's elastic fibre stain. Thrombus appeared to increase in proportion to time of electrification.

FIG. 14. Microscopic finding of 3 V 5 minutes negative charge after clamp.

FIG. 15. Microscopic finding of 3 V 20 minutes negative charge after clamp.

FIG. 16. Microscopic finding of 3 V 30 minutes negative charge after clamp. Thrombus did not form in contact with clamed intima as well as the control case of no injury, but destruction of blood vessel tissues was observed.

FIG. 17. Venograph; Occluded case (Control). No electrification two hours after artificial blood vessel transplantation in the inferior vena cava.

FIG. 18. Venograph; Occluded case (Pt-plate electrode). 6 V, 2 hours negative charge after artificial blood vessel transplantation in the inferior vena cava.

FIG. 19. Venograph; Patent case (Pt-plate electrode). 6 V, 2 hours negative charge throughout artificial blood vessel transplantation in the inferior vena cava.

FIG. 20. Venograph; Occluded case (Control, Platinum coilel ectrode). No electrification.

FIG. 21. Venograph; Patent case (Platinum coil electrode). 6 V, 2hours negative charge after artificial blood vessel transplantation in the inferior vena cava.

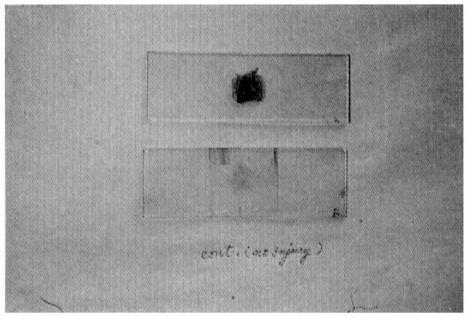

FIG. 5

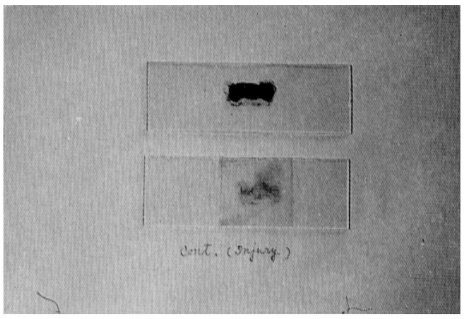

FIG. 6

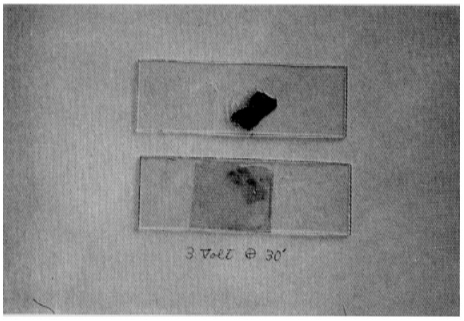

FIG. 7

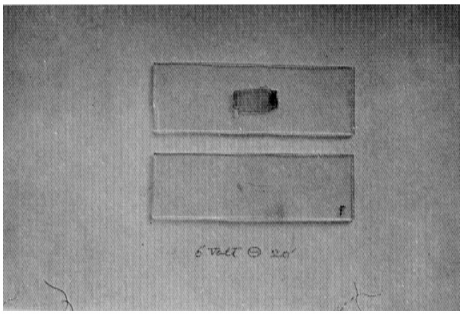

FIG. 8

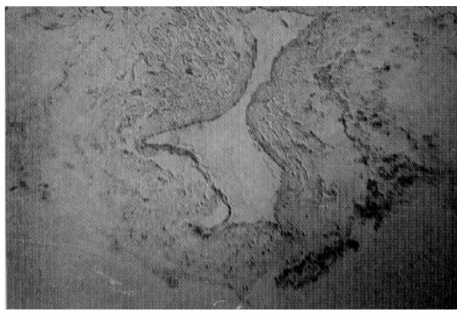

FIG. 9

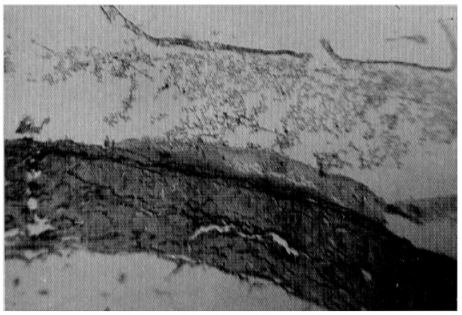

FIG. 10

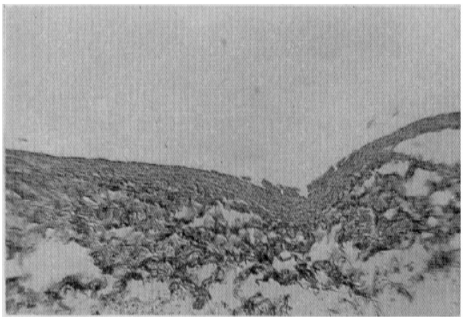

FIG. 11

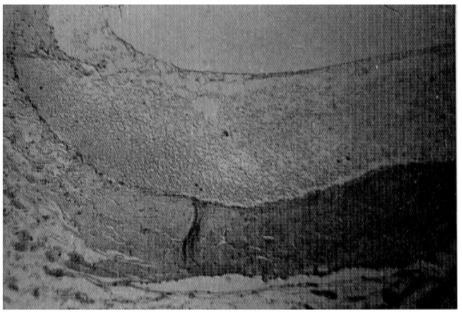

FIG. 12

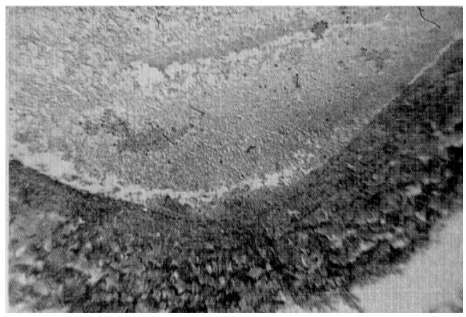

FIG. 13

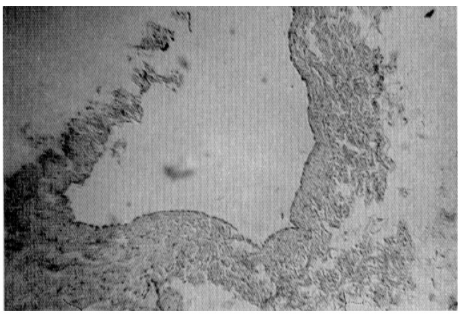

FIG. 14

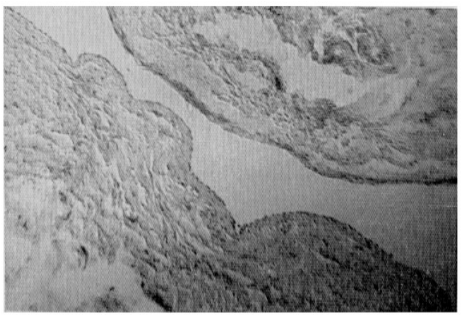

FIG. 15

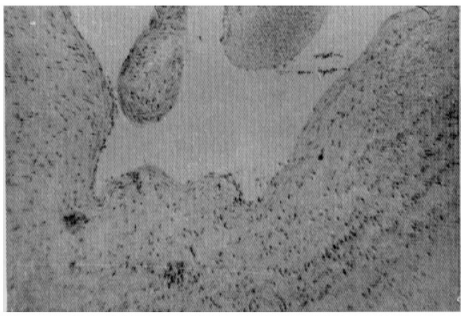

FIG. 16

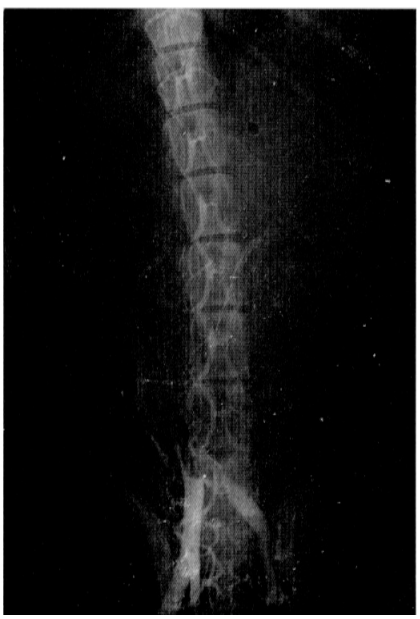

FIG. 17

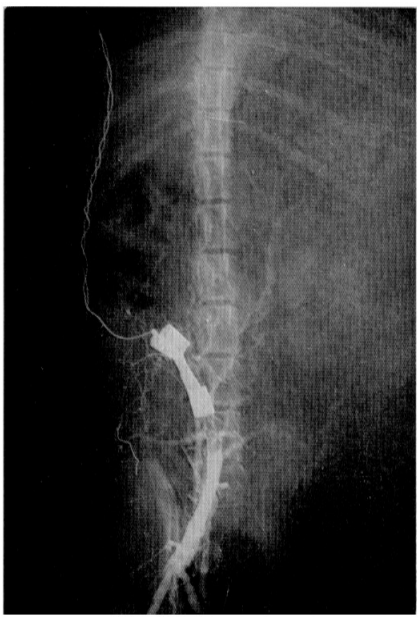

FIG. 18

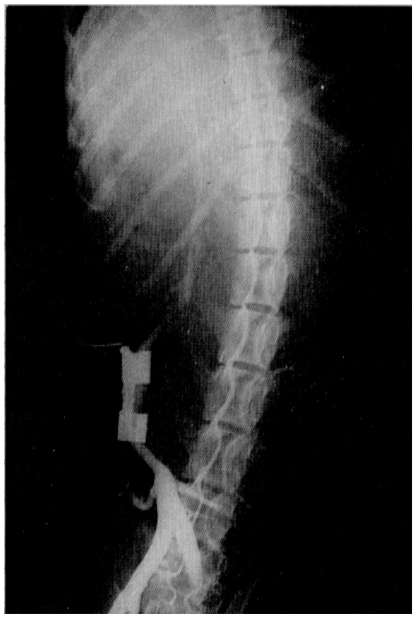

FIG. 19

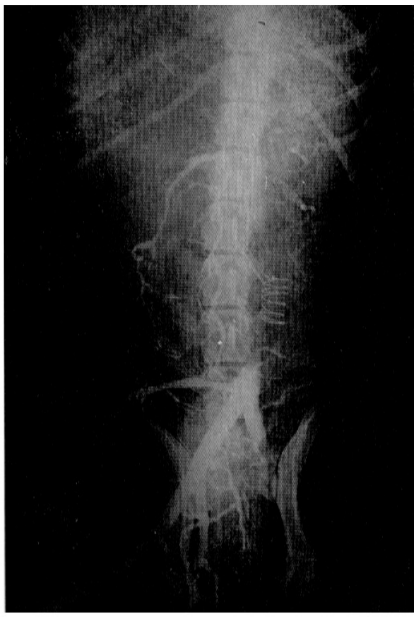

FIG. 20

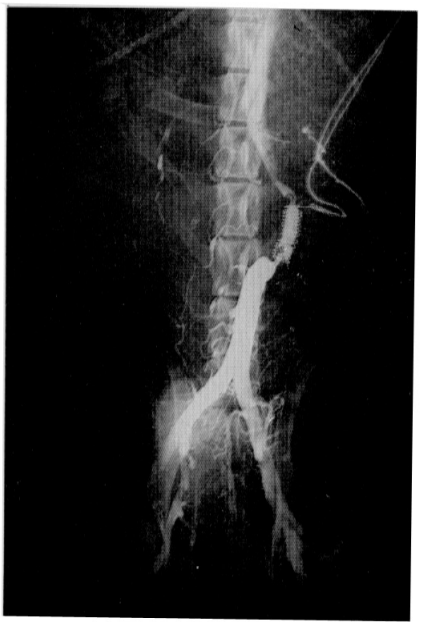

FIG. 21