Electrically Induced Arterial Thrombosis in Acutely and Chronically Instrumented Dogs

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Abstract

A technique for the electric induction of arterial thrombosis was developed and evaluated. The phase 1 acute study consisted of a single stimulation of eight vessels in three dogs. Stimulus parameters were 0.5 or 0.7 mA for 3 hours from a constant current power supply through a positive potential solid gold electrode in contact with the arterial adventitia. Electromagnetic flow measurements and microscopic examination of the stimulated arteries and control segments were performed from 2 to 14 days after stimulation. The phase 2 chronic study consisted of the implantation of stimulating electrodes, flow transducers, and hydraulic occluders in test and control vessels of three dogs. The right carotid artery in two dogs and the right iliac artery in one dog were stimulated at 0.7 mA for 1.5 hours, once a week until resting and reactive hyperemic flow values and angiography indicated significant or total occlusion. Thrombi were formed in stimulated segments, with total occlusion occurred in two of the three chronic stimulations. Mean flow and reactive hyperemia decreased progressively with time in the implanted dogs. Some thrombosis also occurred under all of the implanted flow transducers.

Key Words

Thrombosis; electrically induced thrombosis; reactive hyperemia; electromagnetic flow transducer; peripheral vascular function; hydraulic occluder

Introduction

A technique for selectively inducing arterial thrombosis in test animals has application in many research studies and equipment evaluations. Among these are studies of coronary insufficiency on stressed animals; evaluation of external Doppler (such as B-mode) ultrasonic

scanning techniques, elucidation of the processes of endothelialization, recanalization, and the pathogenesis of atherosclerosis[1]; and evaluation of new angiographic techniques. In addition, secondary effects of thrombosis, such as peripheral redistribution of blood flow and development of collateral vessels may be studied. Electrically induced thrombosis offers the investigator the opportunity to selectively place an arterial thrombus that microscopically and angiographically resembles natural disease. Although electric stimulation of blood vessels in vivo, with production of thrombi, has been performed before[2, 3, 4], no previous attempts have been made to implant stimulating electrodes and flow transducers for controlled progressive thrombus induction. In addition, poor reproducibility of former techniques has been overcome by the development of a more suitable electrode and stimulation source.

Material and Methods

Electrodes

Lamb[3] successfully produced a thrombus with a gold electrode attached to the outside of a blood vessel. For our study, solid gold electrodes with a surface area of 9 mm^2 were used. The 3-mm long sections were cut from a ribbon of solid gold that was 3-mm wide and 0.203 thick (Western Gold and Platinum Co., San Francisco, CA). The assembly of an electrode suitable for acute studies and chronic implantation is shown in Figure 1. The reference electrode was a silicone insulated multi-stranded stainless steel wire sewn into the adjacent muscle.

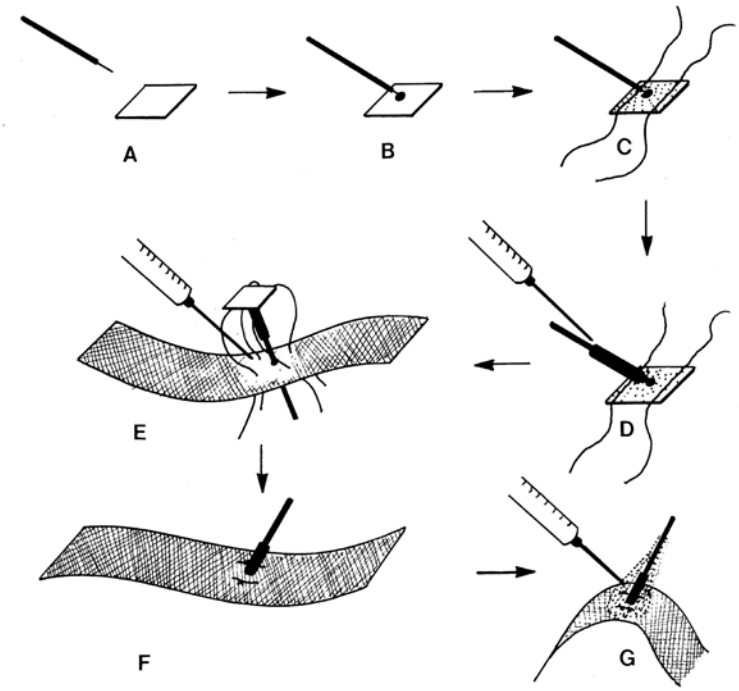
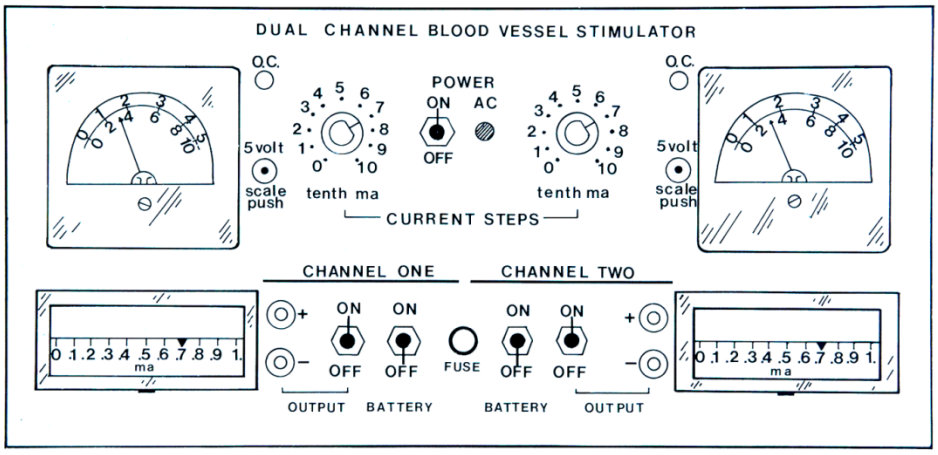


Fig. 1 Construction of the implantable stimulating electrode requires a solid gold plate (3 by 3 by 0.203 mm), Silastic insulated wire (multi-stranded stainless), reinforced Silastic sheeting (Dow Corning #501-3, 7 by 1.5 mm), two 5-0 silk sutures, epoxy (Armstrong A2), 1.0 ml syringe, medical grade adhesive silicone (Dow Corning 891), and a piece of shrink tubing (1 cm long with a diameter sufficient to produce a sleeve around the lead wire). The lead wire is brazed to the gold (**a**) and then dipped into an ultrasonic cleaner to remove remaining flux (**b**). The silk sutures are placed on top of the gold plate and fastened with epoxy (**c**). The shrink tubing is brought down over the lead wire to the gold plate, injected with silicon adhesive, and then shrunk tight (**d**). The lead wire is then passed through the Silastic sheeting, and the silk threads are sewn through the sheeting and tied (**e**). Additional silicone adhesive is applied to the Silastic sheeting as the electrode is pulled tight by tying (**f**). The tread ends and tubing are then covered with sufficient silicone adhesive to insulate and provide strain-relief (**g**). The other end of the lead wire is soldered to a connector pin, and secured with shrink tubing and silicone adhesive.

Stimulation Source

A dual channel blood vessel stimulator was fabricated (Fig. 2). Each channel delivered 0 to 1.0 mA in steps of 0.1 mA, with voltage compliance (range) of 0 to 8 V. Calibration allowed for current regulation of +/- 0.1%. A high-impedance constant current source was obtained by placing the load in the feedback loop of an inverting operational amplifier. Isolation was achieved by use of rechargeable batteries as a power source. The probe electrode in contact with the vessel was at positive potential, and an electrode attached to the adjacent muscle was at negative potential, as per Lamb[3].



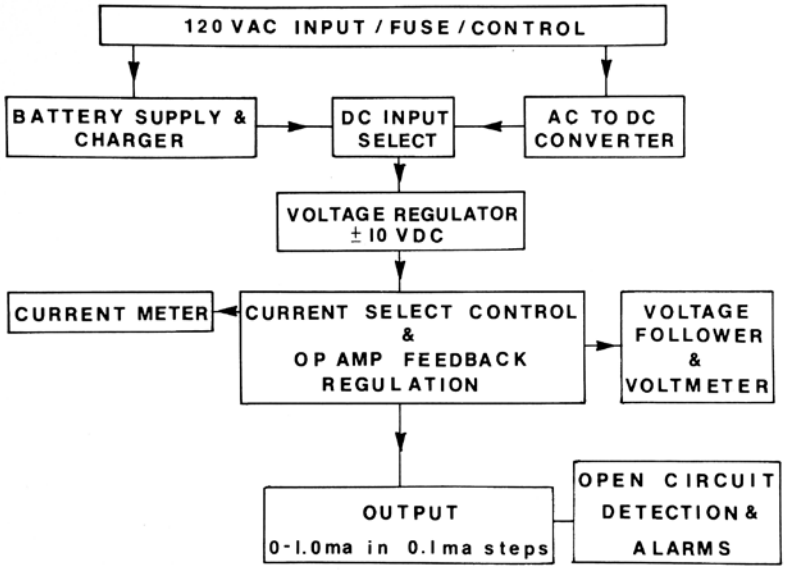


Fig. 2 Front panel and block diagram of the stimulator. Current is set between 0 and 1.0 mA with the current step switch. The current meter reads the current selected in mA, and the voltmeter reads the voltage. The O.C. (open circuit) light and audible alarm warns of an open circuit or defective electrode.

Phase 1 Acute Study

Eight vessels in three dogs were stimulated according to procedures outlined in Table 1. The dogs weighed from 19 to 25 kg and were anesthetized initially with 1.0 ml/7 kg body weight with Innovar-Vet intramuscularly. (Innovar-Vet contains 0.4 mg fentanyl and 20 mg droperidol). Anesthesia was maintained with 2 ml of 50 mg/ml sodium pentobarbital administered as needed intravenously through a catheter maintained patent with normal saline. Each dog had an endotracheal tube connected to a positive-pressure demand respirator.

All procedures were performed in sterile field. After a carotid artery was exposed, an electromagnetic flow transducer (Zepada Instruments, Seattle, WA) and stimulating electrode were placed proximal and distal, respectively, along the vessel (Fig. 3). In addition, a reference electrode was sewn into the muscle adjacent to the vessel, and a 1-0 silk thread was passed under the vessel and momentarily tightened for calibration of the flow transducer at zero flow.

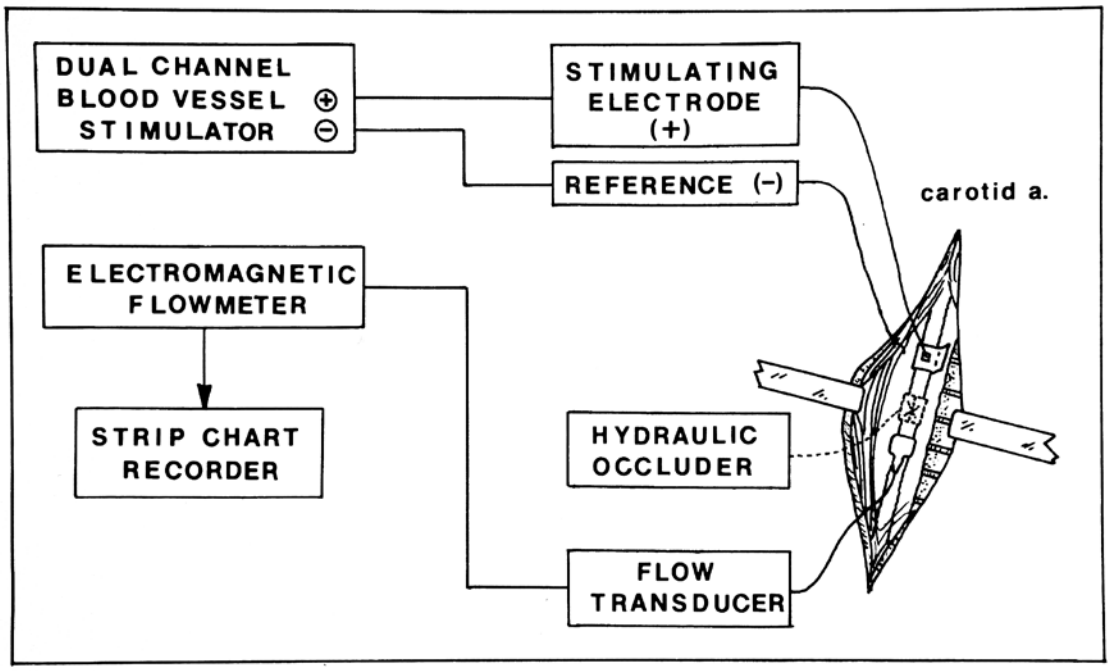


Fig. 3 Arrangement of the instrumentation during the acute study. Current levels of 0.5 and 0.7 mA were chosen for the study. The flowmeter (Zepada Instruments, SWF 4-RD) was connected to the flow transducer only when the stimulator leads were disconnected. The recorder displayed phasic and mean flow. The stimulator reference electrode was sewn into the adjacent muscle. During the chronic study, a miniature hydraulic occluder was placed between the transducer and stimulating electrode.

After measurement of initial flow, the flow transducer leads were disconnected from the flowmeter and the stimulating electrode leads connected to the power supply. After 3 hours, the stimulator leads were disconnected and the flow transducer leads were reattached for measurement of final flow. Manual occlusion of the vessel was not performed after stimulation because of the risk of dislodging a newly formed thrombus. All electric devices were then removed from the dog. Three of the stimulated arteries were removed before closing (specimens 127, 197, 199) while the other five were removed at 2, 4, 6, 10 and 14 days after stimulation. With each resection and adjacent section of artery was obtained for control purposes. Sections were placed in 10% formaldehyde and saline solution.

Phase 2 Chronic Study

Four dogs were implanted with flow transducers, miniature hydraulic occluders, and stimulating electrodes. The carotid arteries were used in two of the dogs, while the iliac arteries were utilized in the other two dogs. In one dog, flow signal was lost from the iliac artery, and this dog was excluded from the study. In all chronically instrumented dogs, the right artery received the flow transducer, hydraulic occluder, and stimulating electrode (proximal to distal, respectively), while the left artery (serving as a flow control) received a flow transducer. For the implantation procedure, the dogs were anesthetized as in the acute study and each received 1.0 g of methicillin intravenously during the operation. All leads were routed subcutaneously and were brought out through the skin at the dorsal neck.

Starting with the eight day postoperatively each dog was stimulated according to the procedure outlined in Table 2. Each dog was stimulated no more than once per week, with phasic and mean flows recorded before and after stimulation. A 30-second “occlusion zero” with measurement of reactive hyperemia was performed three times just before stimulation. To accomplish this, the vessel was briefly occluded with a hydraulically operated cuff around the vessel as shown in Figure 4. This is a variation of the one described by Debley[5].

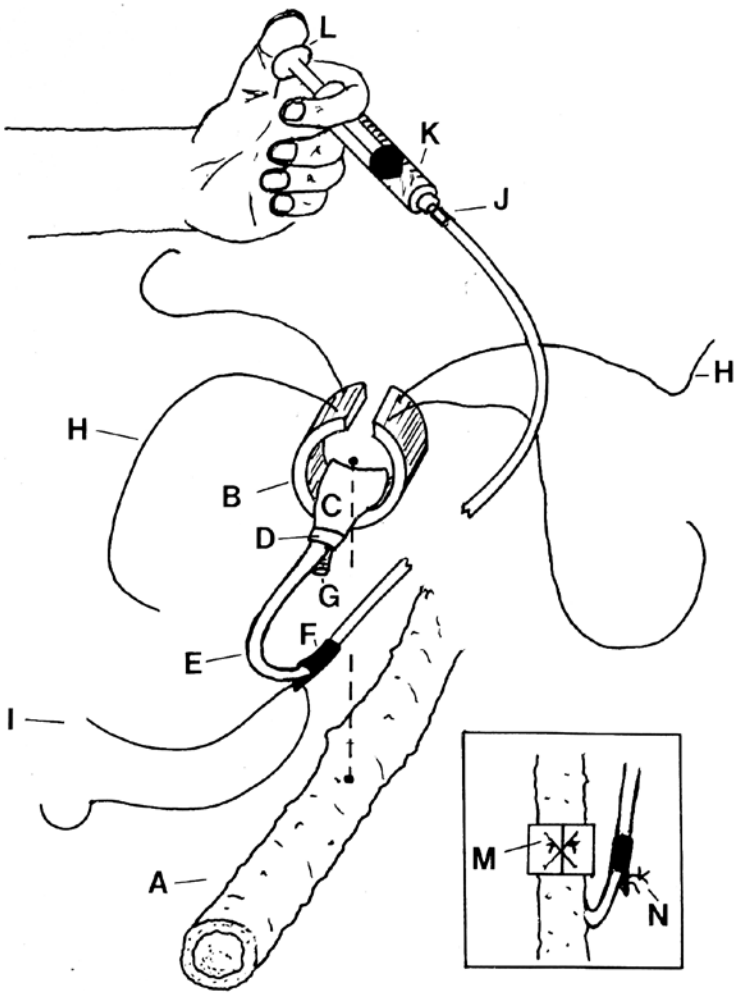


Fig. 4 The hydraulic occluder shown here allowed momentary occlusion of the blood vessel ("occlusion zero"). This is necessary for calibration of the electromagnetic flow probe and measurement of reactive hyperemia. The following are shown: artery (**a**), 7 mm section of Tygon tubing with lumen diameter of 6 mm (**b**), expanded Tygon tubing balloon (**c**), 3 mm section of intravenous tubing (**d**), 1-m section of the 2 mm diameter Tygon tubing (**e**), 1.0 cm section of Tygon tubing of sufficient diameter to slip over **e** (**f**), heat-sealed end of **e** (**g**), 3-0 Mersiline (**h**), 5-0 silk suture (**i**), cut and polished 16-gauge syringe needle (**j**), 30-ml syringe filled with sterile water (**k**), application of continuous pressure (**l**), cross-tie of Mersiline threads (**m**), and fastening down of the occluder (**n**). The balloon was made by expanding the end of **e** in a beaker of hot water. **F** is attached to **e** with a drop of cyclohexanone. Before heat-

sealing the end of **e**, the occluder was gas-sterilized and filled with sterile water. The other end of the tube was sealed when the syringe was not attached.

Stimulations were performed while the dog rested unanesthetized in a Pavlov sling. Once flow and hyperemic response were significantly reduced, angiography was performed. An angiographic catheter (Cordis Ducor 7F) was inserted through the femoral artery and was passed up to the brachiocephalic artery for injection of contrast medium (Hypaque) into the carotid arteries. For the iliac-implanted dog, the catheter was inserted through the carotid artery and passed down to the abdominal aorta. Roentgen cinematography at 60 frames per second was done, along with video storage on a disk recorder (providing immediate playback capability) and tape recorder (permanent log).

All dogs were monitored daily for general health and were under the supervision of the facility's doctor of veterinary medicine.

Results

Phase 1 Acute Study

The amount of lumen obstruction caused by thrombus formation in the test segments varied from 10 to 100% (Table 1). The thrombus formed in each test segment was a platelet aggregation or "white thrombus." Endothelialization of the thrombus occurred in specimens 325, 157, 200 and 195. In all specimens, the original endothelium underlying the thrombus was denuded. The elastica had a straightened appearance and was often fragmented. The media and adventitia displayed various degrees of necrosis, fibrosis, hemorrhage and granulation. These findings suggest that the endothelium is injured as a result of either direct or indirect changes in adventitial blood supply. The size of the thrombus was not related to the stimulus current.

Table 1 Phase 1 Acute Study Procedures and Resulting Thrombus Obstruction in Test Arterial Segments*

Specimen	Dog	Artery	Current (mA)	Voltage (V)	Interval (days)**	Obstruction (%)***
197	373	R femoral	0.5	3.3	0	80
199	373	L femoral	0.5	3.6	0	100
127	375	R femoral	0.5	3.8	0	10
326	375	L carotid	0.7	3.4	2	30
325	375	R carotid	0.7	4.2	4	50
157	379	L femoral	0.7	3.6	6	U
200	379	R carotid	0.7	3.5	10	20
195	379	L carotid	0.7	3.5	14	U

* All segments were stimulated for 3 hours.
** Number of days between stimulation and resection.
***Visual estimate of lumen obstruction from fixed section.
U=Uncertain (poor section).

A low-power cross-sectional view of specimen 325 (right carotid artery) is shown in Figure 5A.

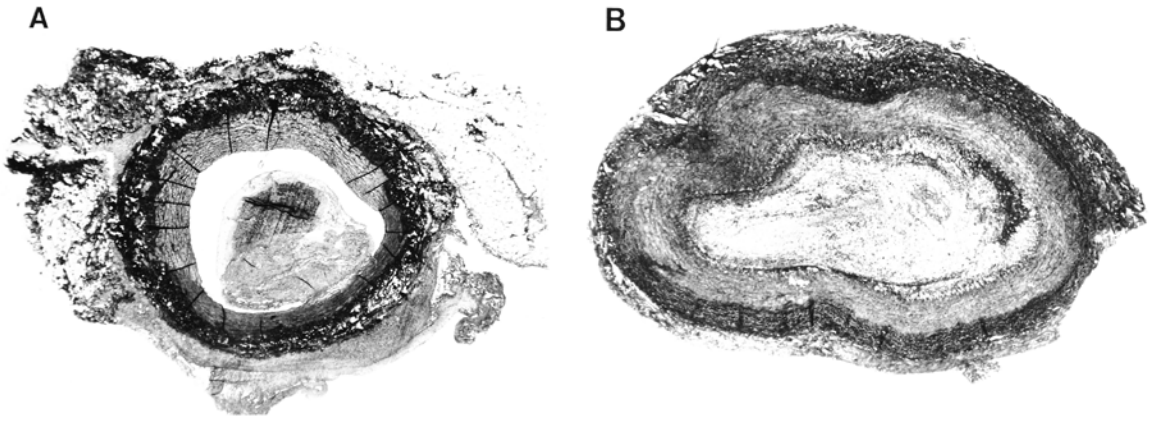


Fig. 5 **A**, Cross sectional view of specimen 325. This phase 1 acute study shows considerable obstruction of the arterial segment by white thrombus. Thrombus is attached to the lumen wall under the site where the gold electrode was in contact with the vessel. **B**, Cross-sectional view of specimen 329. This phase 2 chronic study shows total occlusion of the arterial segment by a white thrombus, with slit-like recanalization and proliferation of the intima and media.

An initial concern, that the flow transducer might interfere electrically with the process of thrombosis, was investigated with specimens 197 and 199. Specimen 197 was stimulated with the transducer on continuously, while specimen 199 was stimulated with the transducer off. Gross comparison (that is, thrombus vs. no thrombus) revealed little difference between specimens.

Phase II Chronic Study

The amount of lumen obstruction caused by thrombus formation in the test segments varied from 20 to 100% (Table 2). In each test segment, a white thrombus with various degrees of slit-like endothelialization recanalization developed. Moreover, there was prolifera-

tion of the intima and media in each specimen, as well as some fibrosis and necrosis of the adventitia.

Table 2 Phase 2 Chronic Study Procedures and Resulting Thrombus Obstruction in Test Arterial Segments

Specimen	Dog	Artery	Current (mA)	Voltage (V)*	Total Implantation (days)	Total Stimulations **	Obstruction (%)
226	376	R carotid	0.7	2.5-3.5	35	2	20
321	373	R carotid	0.7	2.0-2.7	37	2	100
329	343	R iliac	0.7	2.0-2.4	36	4	100

* Range of voltages observed throughout implantation. (In a constant current power supply, the voltage is compliant, and will vary with changes in the tissue impedance.)
** Stimulation time was 1.5 hours; and occurred at week 2 and 3 for specimens 226 and 321; and week 2, 3, 4 and 5 for specimen 329.

The slit-like recanalization of specimen 329 is shown in Figure 5B (right iliac artery). All of the control specimens (acute and chronic study) were normal except for a small area of absent endothelium which was probably caused by trauma during resection and fixation.

Mean flow and reactive hyperemia (Fig. 6) decreased with time in the stimulated segments. The significance of these findings is limited by the sample size. However, the decreased hyperemia was consistent with the development of collateral vessels observed on angiography.

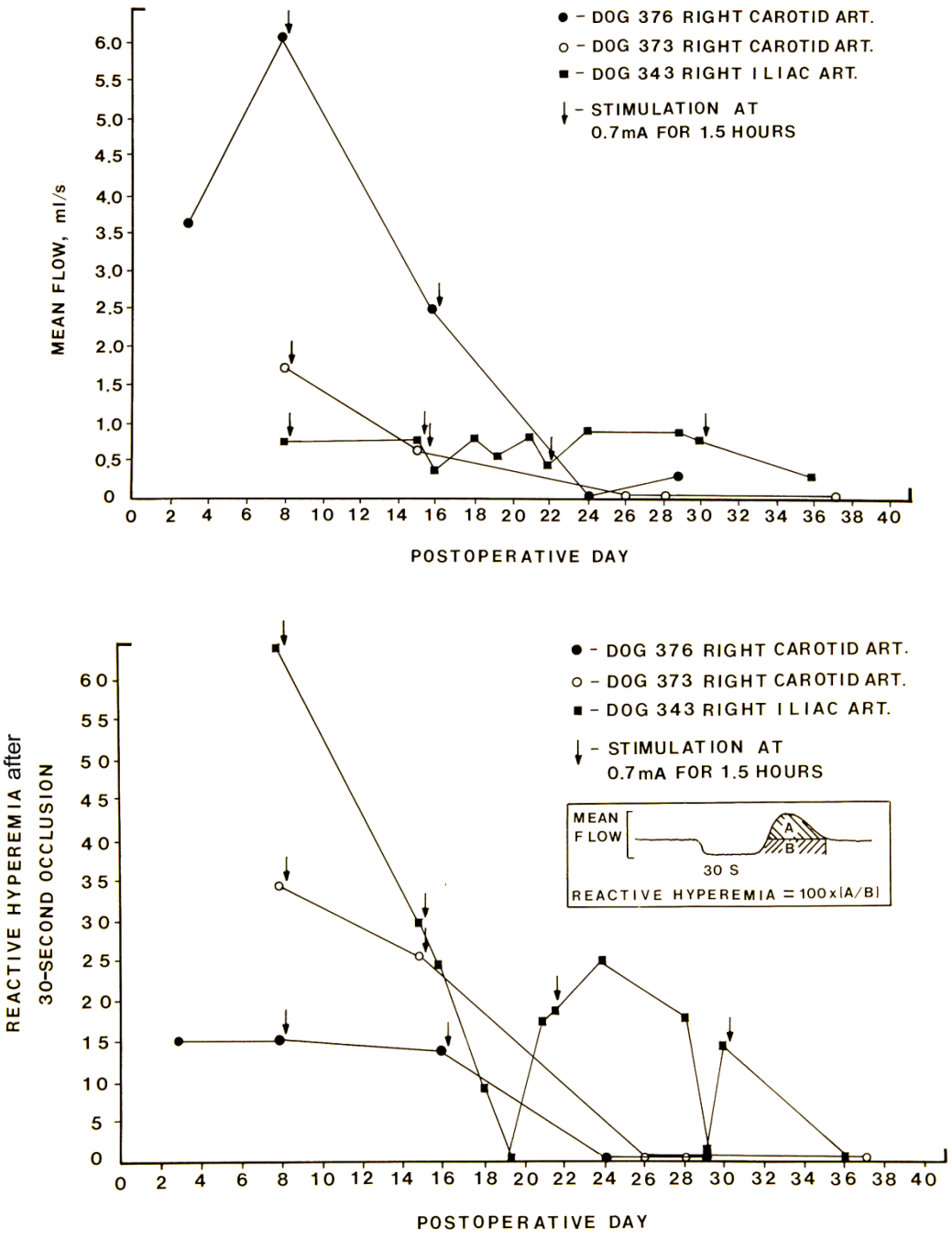


Fig. 6 **Top Panel**, Mean flow progressively decreased with time in chronically instrumented dogs. **Bottom Panel**, Reactive hyperemia also decreased in these dogs, as would be expected because of collateral flow development.

The flow changes in the right iliac artery of dog 343 (specimen 329) and the resulting stenosis of four stimulations are shown in Figure 7, and the corresponding angiogram in Figure 8.

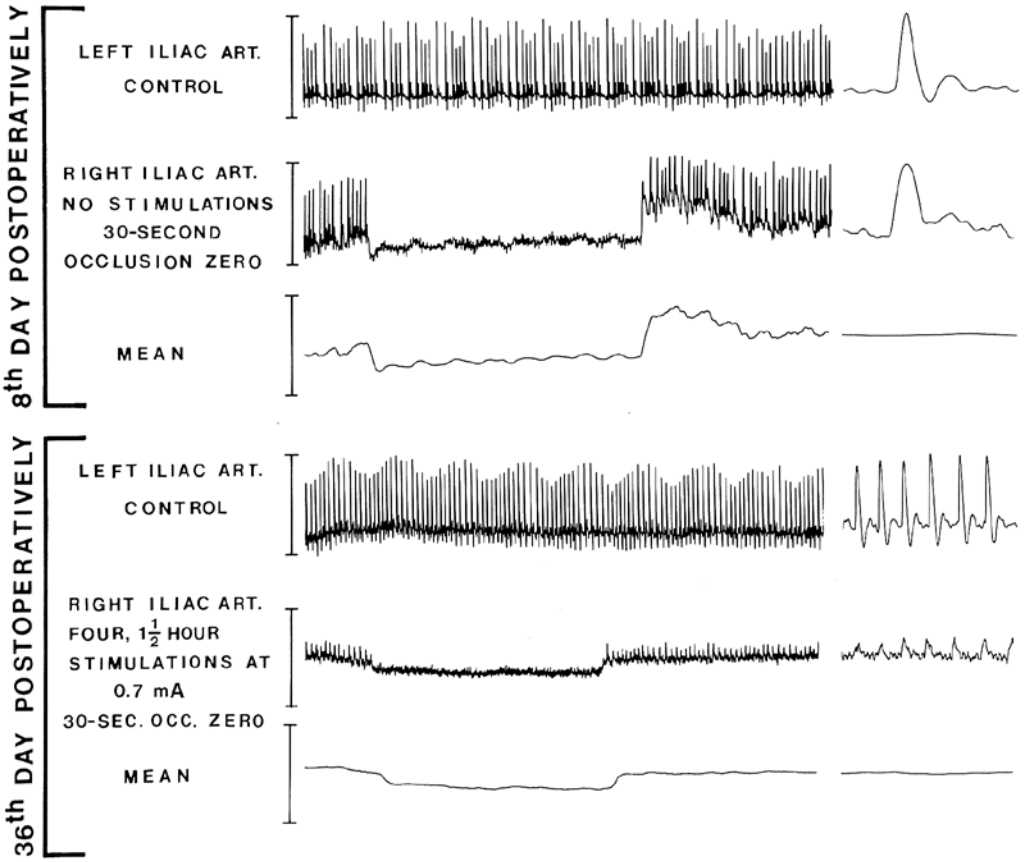


Fig. 7 Mean flow and hyperemic response in dog 343 (specimen 329) at 8 and 36 days after implantation. Changes in flow wave form can be seen in the expansion of the tracings on the right. The vertical scales represent approximately 7.8 ml/s full scale.

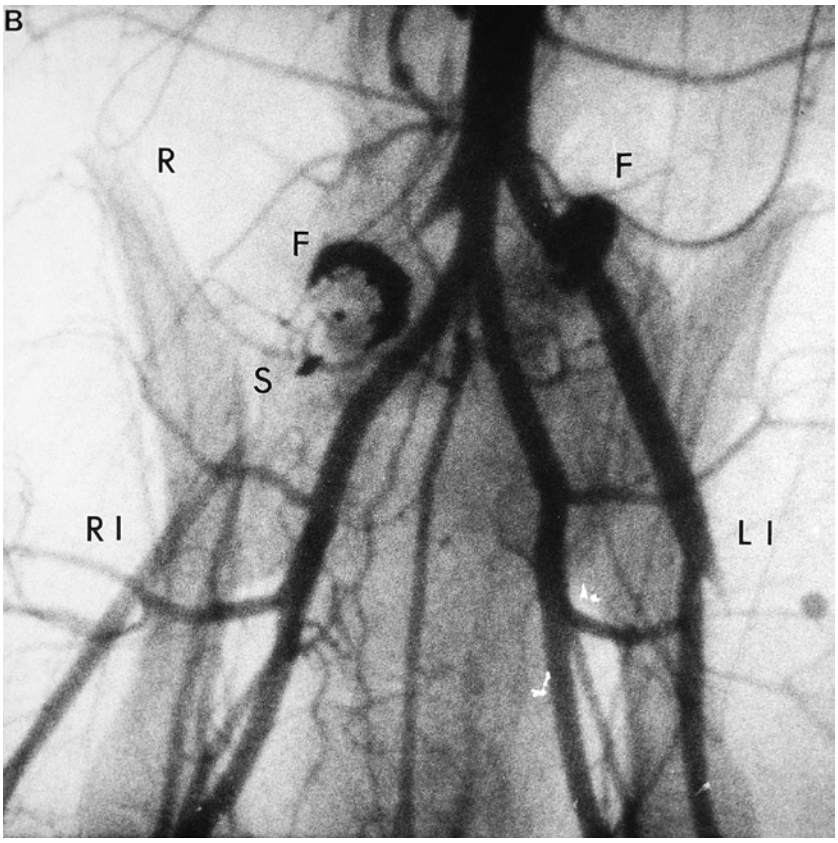


Fig. 8 Total occlusion of the right common iliac artery in dog 343 is evident. **S** is the stimulating electrode, **R** the reference electrode, **F** the flow transducer coil, **RI** right iliac artery, and **LI** the left iliac artery.

An unexpected findings was the presence of thrombi under the flow transducers on both test and control vessel sides. The flow probe was driven with a constant current (0.5 A) at selected frequencies (80 to 1,000 Hz). The data concerning the six flow transducers implanted in the chronic study are summarized in Table 3.

Table 3 Arterial segments under chronically implanted flow probes*

Specimen	Dog	Artery	Artery Side	Total Implantation (days)	Drive Time (hours)**	Obstruction (%)
224	376	L carotid	control	35	2.77	75
225	376	R carotid	test	35	2.77	25
323	373	L carotid	control	37	1.77	50
322	373	R carotid	test	37	1.77	80
328	343	L iliac	control	36	2.36	5
327	343	R iliac	test	36	2.36	5

* Significant granulation of the adventitia made these specimens recognizable from the others by simply holding the glass up to light.
** Number of hours the flow transducer was on.

Discussion

We have demonstrated the induction of a thrombus by electric stimulation in acute and chronically instrumented dogs. The resulting pathologic changes resemble natural arterial thrombosis both microscopically and angiographically. The induced thrombus may be selectively placed along the vessel and, by repetitive stimulation, may be made to totally occlude the vessel.

Electrodes

Cowan and Monkhouse[2] produced thrombosis in the mesenteric vessels of mice by use of platinum electrodes. Chopra and colleagues[6] studied the deposition of thrombus on

metal electrodes inserted through side branches into the carotid and femoral arteries of dogs. They found that copper, nickel, gold, and platinum electrodes showed a measurable deposition of thrombus along the length of the electrode. All of these metals have positive resting potentials at the metal blood interface. Other metals with negative resting potential were unable to cause thrombus formation. Our successful use of gold electrodes attached to the outside of the vessel confirms the approach used by Lamb[3] and others.

Stimulating Source

Lamb[3] and others have used voltage-controlled power supplies for inducing thrombosis. Lamb reported that 2.0 V was a critical voltage below which thrombosis would not occur in femoral veins of dogs. (Above 2.5 V, thrombosis occurred.) Moreover, in vitro, whole blood coagulated on a positive electrode when the electrode potential was 2.0 V. For our study an electrically isolated, high-impedance, constant current stimulus was chosen, and the voltage was complaint based on unknown tissue impedance (Ohm's law).

Cowan and Monkhouse[2] found that, with a negative electrode at the vessel, constriction occurred, and with a positive electrode at the vessel, clumping and thrombosis with constriction occurred. Lamb also used a positive electrode at the vessel.

Mechanism

Sawyer and colleagues[4, 7, 8, 9] have considered the surface charge of blood cells and have attempted to correlate this charge with thrombosis. Constantinides and Robinson[10] examined the endothelium of rat arteries after exposing the arteries to solutions of varied pH, osmolarity, and temperature. The endothelial cell membranes withstood perfusion with solutions of pH 11.1, osmolarity changes from 0 to 3,000 osmol, and temperatures from 5 to 42 degree C, and exposure to cyanide and arterial clamping for 16 minutes. Exposure to solution less than pH 4.2 caused breaks in the endothelial lining. Robertson and Khairallah[11] investigated the effects of sudden changes or arterial permeability to circulating macromolecules

and the initiation of vascular disease. Salzman[12] described two principle hypotheses concerning the genesis of thrombosis. The first was that thrombosis results from a hypercoagulability state of the blood which under favorable conditions, leads to clotting and accumulation of fibrin. The second hypothesis was that the primary event in thrombosis is a vascula abnormality, such as a break in the continuity of the endothelium. Development of a fibrin clot is more typical when flow or fluid shear is small, such as in peripheral veins or behind a stenotic mitral valve. Red blood cells are trapped in the fibrin network and hence the appearance of a red thrombus. In areas of high flow such as in arteries, either platelet or white thrombi develop. Recently, Ross and Glomset[1] reviewed the pathogenesis of atherosclerosis and the role of experimental injury techniques. They discussed the process of arterial smooth muscle proliferation that accompanies endothelial injury. The data from our study confirms the existence of this proliferation process.

The thrombi produced from electric stimulation are platelet aggregations. The most probable explanation is that hydrolysis occurs at the positive electrode, causing the pH to decrease below the critical level of 4.2 noted by Constantinides and Robinson[10]. This results in denuding of endothelium and exposure of underlying collagen to the circulating blood. Platelets attach to the collagen and undergo a “release reaction” whereby ADP, epinephrine, ATP, 5-hydroxytryptamine, platelet factor 3, and antiheparin factor are released. ADP promotes additional aggregation of platelets, and a thrombus develops. Proliferation of the media and intima may be the result of interaction of “chalones.” These are postulated inhibitors of stem cells of the media and intima produced by smooth muscle cells[1]. Chalones probably provide “population equilibrium” in the normal artery by feedback control.

Conclusion

A thrombus may be artificially produced in any artery that can be sufficiently isolated for attachment of a stimulating electrode. Artificial stenosis of blood vessels has been produced

by mechanical devices[13], chemical techniques[14], and by simply tying off the appropriate vessel. Applications have included studies or peripheral flow and hyperemia[15, 16] and coronary insufficiency[17]. The clearest advantage of electrically induced thrombosis is that the induced thrombus resembles natural disease both microscopically and angiographically.

Supplemental

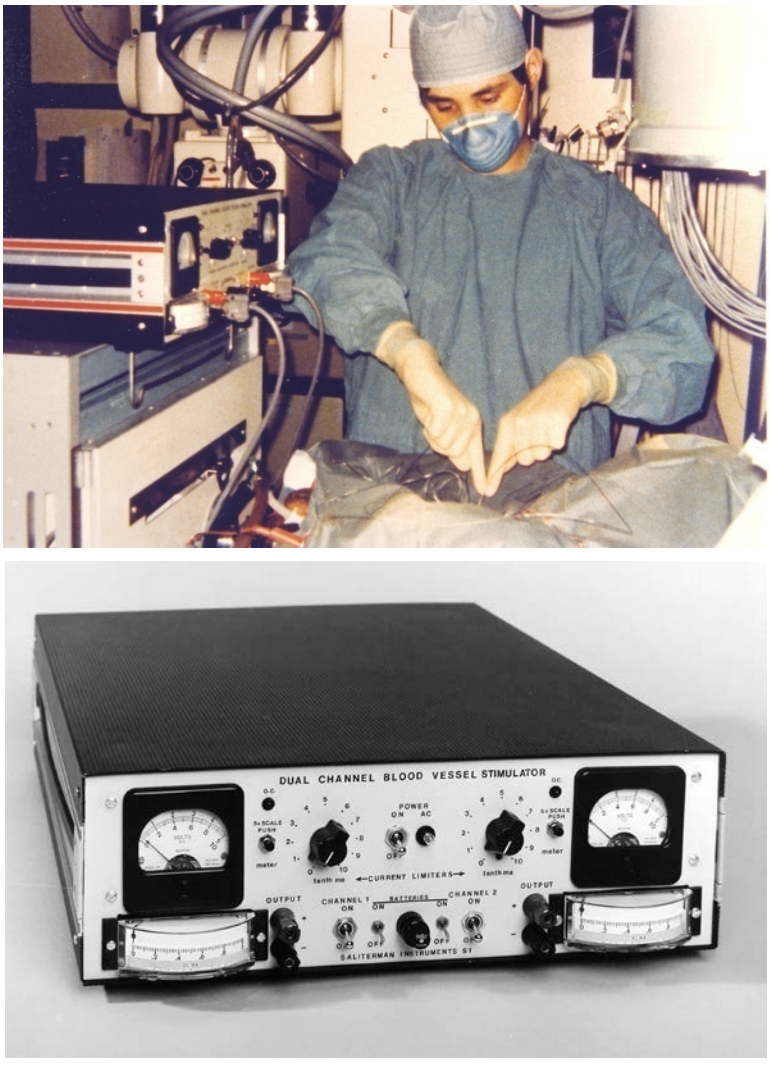


Fig. 9 **Top**, Attachment of stimulating electrode and flow probe. **Bottom**, Constant-current power supply.

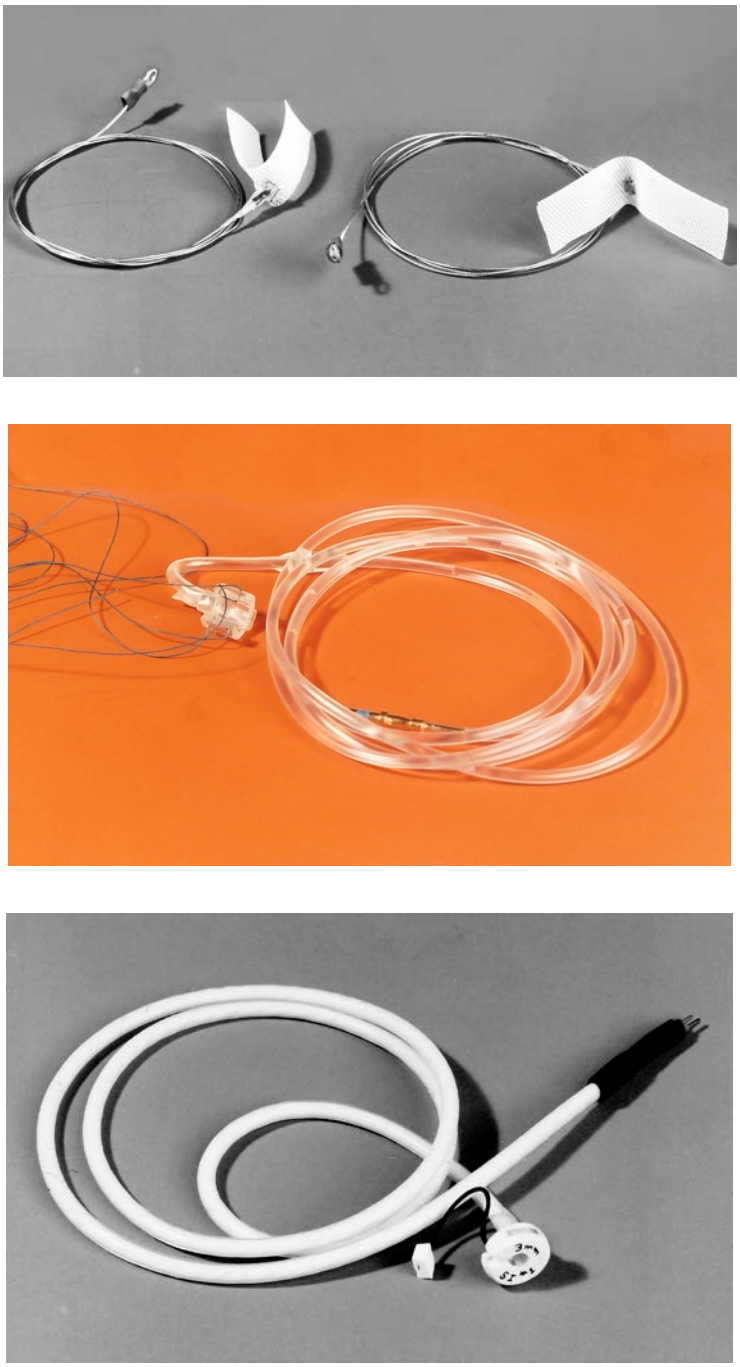


Fig. 10 Top, Stimulating electrode. **Middle**, Hydraulic occluder. **Bottom**, Probe-style stimulator (in development).

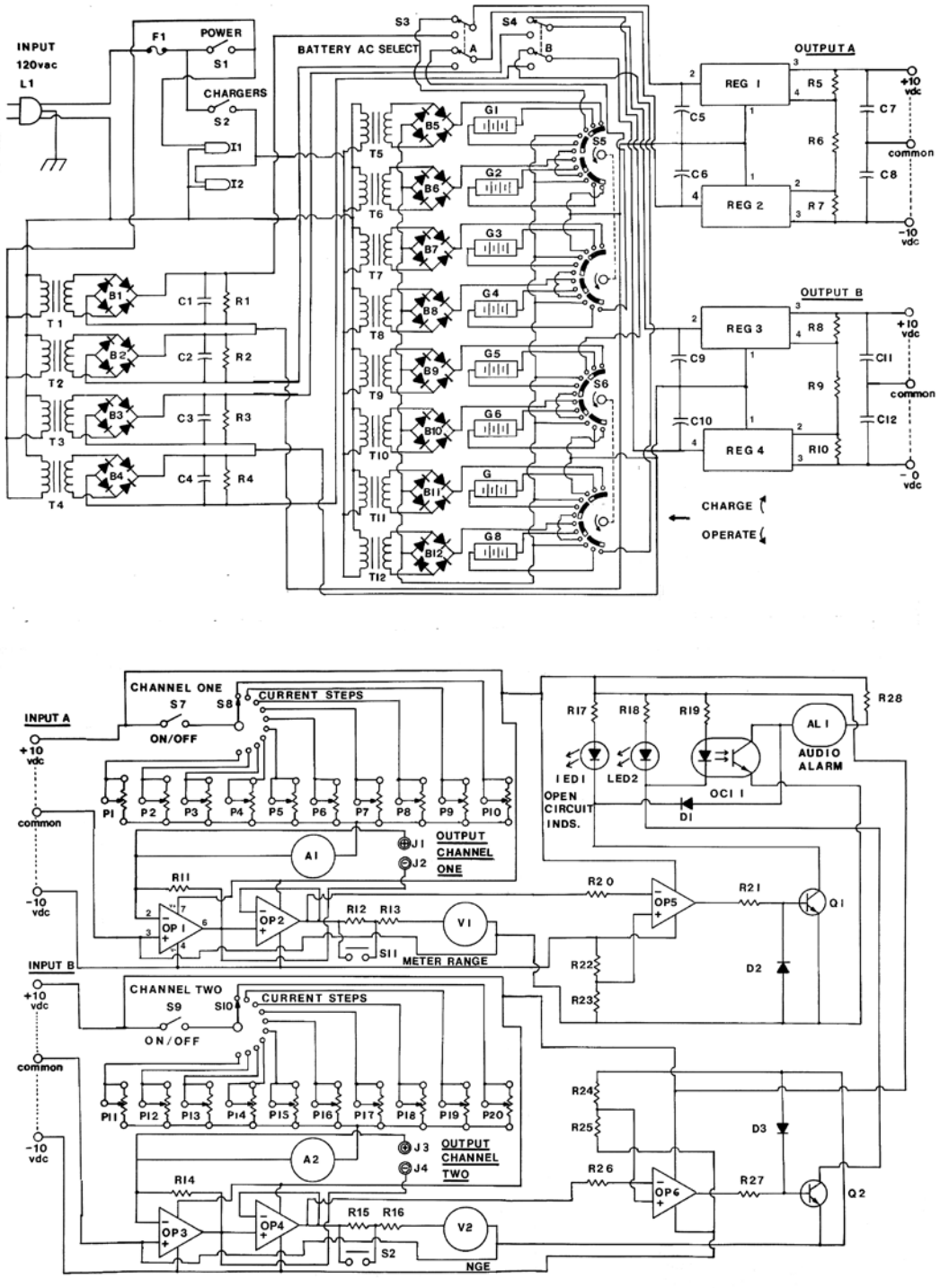


Fig. 11 **Top,** Charger and regulated DC input circuits. **Bottom**, Current selection, high-impedance operational amplifier feedback drive circuits, and alarm circuits[18].

References

1. Ross, R. and J.A. Glomset. The pathogenesis of atherosclerosis. N. Eng. J. Med. 294:369-377 and 295:420-425. 1976.

2. Cowan, C.R. and F.C. Monkhouse. Studies on electrically induced thrombosis and related phenomenon. Can. J. Physiol. and Pharm. 44:8810886. 1966.

3. Lamb, J.C., J.P. Isaacs, W.L. Bloom, and D.S. Harmer. Electrical thrombosis of blood vessels: A voltage dependent phenomenon. Am. J. Physiology. 208(5):1006-1008. 1965.

4. Sawyer, P.N., B. Deutch and J.W. Pate. The relationship of bioelectric phenomena and small electric currents to intravascular thrombosis. Thrombosis and Embolism, Basel: Benno Schwabe and Co. 1955.

5. Debley, V.G. Miniature hydraulic occluder for zero blood flow determination. J. Appl. Physiol. 31(1):138-139. 1971.

6. Chopra, P.S., S. Srinivasan, T. Lucas and P.N. Sawyer. Relationship between thrombosis on metal electrodes and the position of metal in the electromotive series. Nature 215:1494. Sept. 30, 1967.

7. Sawyer, P.N., J. H. Reardon and J.C. Ogoniak. Irreversible electrochemical precipitation of mammalian platelets and intravascular thrombosis. Proc. N.A.A. 53:200-207. 1965.

8. Sawyer, P.M. and S. Srinivasan. Studies on the biophysics of intravascular thrombosis. Am. J. of Surg. 114:42-60. 1967.

9. Sawyer, P.N. and S. Srinivasan. The role of electrochemical surface properties in thrombosis at vascular interfaces: Cumulative experience of studies in animals and man. Bull. N.Y. Acad. Med. 48(2):235-256. 1972.

10. Constantinides, P. and M. Robinson. Ultrastructure injury of arterial endothelium I. Effects of pH, osmolarity, anoxia and temperature. Arch Path. 88:99-112. Aug 1969.

11. Robertson, A.L. and P.A. Khairallah. Arterial endothelia permeability and vascular disease. Exp. and Mol. Path. 18:241-260. 1973.

12. Salzman, E.W. The events that lead to thrombosis. Bull. N.Y. Acad. Med. 48(2)225-234. 1972.

13. Young, D.F., N.R. Cholvin and A.C. Roth. Pressure drop across artificially induced stenosis in the femoral arteries of dogs. Cir. Res. 36:735-743. June. 1975.

14. Carlson. E.L., J.R. Cant and H.V. Sparks. Coronary blood flow of unanesthetized dogs with experimental artery disease. Cardiovascular Res. 7:789-797. 1973.

15. Kontos, H.A., H.P. Mauck, Jr. and J.L. Patterson, Jr. Mechanism of reactive hyperemia in limbs of anesthetized dogs. Am. J. Physiol. 209(6):1106-1114. 1965.

16. Storen G. and S. Akre. Changes in blood flow and vascular volume in skeletal muscle during reactive hyperemia with free flow or with artificial stenosis, Scan. J. Clin. Lab. Invest. 23:109-114. 1969.

17. Khouri, E.M., D.E. Gregg and H.S. Lowensohn. Flow in the major branches of the left coronary artery during experimental coronary insufficiency in the unanesthetized dog. Cir. Res. 23:99-109. 1968.

18. Saliterman, S. Dual Channel Blood Vessel Stimulator Operating Manual, 1976.