somes attached to contaminating rough endoplasmic reticulum in these preparations (*5, 20*).

The labeled RNA obtained from the mitochondria of mitotic cells resembles the RNA sensitive to ethidium of interphase cells (Fig. 2b). There is, however, no RNA resistant to ethidium labeled in the mitochondria of mitotic cells, presumably because RNA synthesis of nuclear origin has been inhibited.

The incorporation of radioactivity in the mitochondrial fraction of mitotic and interphase cells cannot be used to compare the relative amounts of RNA synthesis because the labeling conditions are quite different. The equilibration of the pyrimidine precursor pools with exogenous material is probably quite different in mitotic and interphase cells because in mitotic cells there is no appreciable nuclear RNA synthesis. Also, no attempt was made in these experiments to insure a continuous uptake of exogenous precursor. However, the label in mitochondrial RNA of mitotic cells constitutes a much larger fraction of total cell incorporation than is the case in interphase cells. The radioactivity in the mitochondrial fraction of mitotic cells is therefore not due to incorporation by contaminating interphase cells. Unlike nuclear RNA synthesis, mitochondrial RNA synthesis continues in mitotic cells.

The observation that mitochondrial RNA in mitotic cells is labeled at nearly the same rate as in interphase cells further suggests that radioactive precursors in fact do enter the cell in relatively normal amounts. Thus, the inhibition of radioactive precursor uptake seen at mitosis is due to a true inhibition of RNA synthesis and is not the result of the exclusion of the radioactive isotope.

Two conclusions can be drawn from the results shown in Fig. 2. First, the RNA associated with the mitochondrial fraction and which is sensitive to ethidium bromide is independent of the control mechanisms which inhibit nuclear RNA synthesis at mitosis. Additionally, the complete disappearance of an ethidium-resistant background during the time that nuclear synthesis is inhibited further supports the hypothesis that RNA resistant to ethidium in the mitochondrial fraction is actually of nuclear origin.

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Radio-Frequency Thrombosis of Vascular Malformations with a Transvascular Magnetic Catheter

Abstract. *A tiny magnetic electrode catheter introduced into the human carotid artery has been mechanically and magnetically propelled, with fluoroscopic control, to cranial arteriovenous malformations. Radio-frequency heating of the catheter tip in successive positions occludes abnormal blood vessels.*

Cerebral aneurysms and arteriovenous malformations present a formidable mortality and morbidity in spite of the best existing medical and surgical treatment. All current methods of surgical treatment require an opening in the skull and a transcortical or pericortical approach which in itself may have disadvantages. Indeed some of these conditions cannot be treated by any current technique because of their location or size. We have partly obliterated two extracranial arteriovenous malformations by a new technique that employs a magnetically and mechanically guided intravascular electrode and radio-frequency thrombosis.

The coagulating and hemostatic cutting effects of high-frequency electrical currents have been used in brain surgery since 1926 (*1*) and magnetic guidance of a catheter for selective angiography of the aorta was described in 1951 (*2*). Recently magnetically controlled brain catheters have been used in humans (*3*).

We designed a variety of multipurpose, magnetic-tipped catheters small enough (0.5 mm in diameter) to enter human cerebral arteries. A cylindrical samarium-cobalt magnet (*4*) 0.5 mm in diameter and 1.5 mm in length is

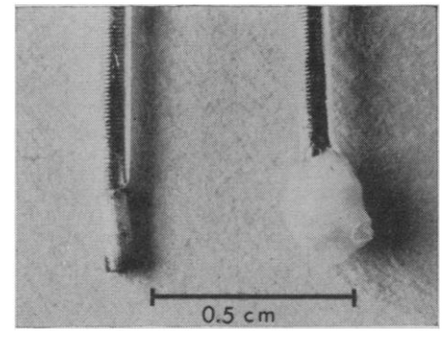
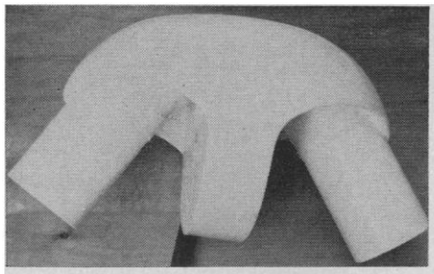


Fig. 1. (Left) Photograph of the transvascular magnetic electrode catheter. (Right) A radio-frequency lesion that has been made by immersing the catheter tip in egg albumin at 70 ma for approximately 5 seconds. The catheter was rapidly moved through the egg white as the lesion was being made to attempt to simulate heat dispersion by blood flow such as might occur in an artery.



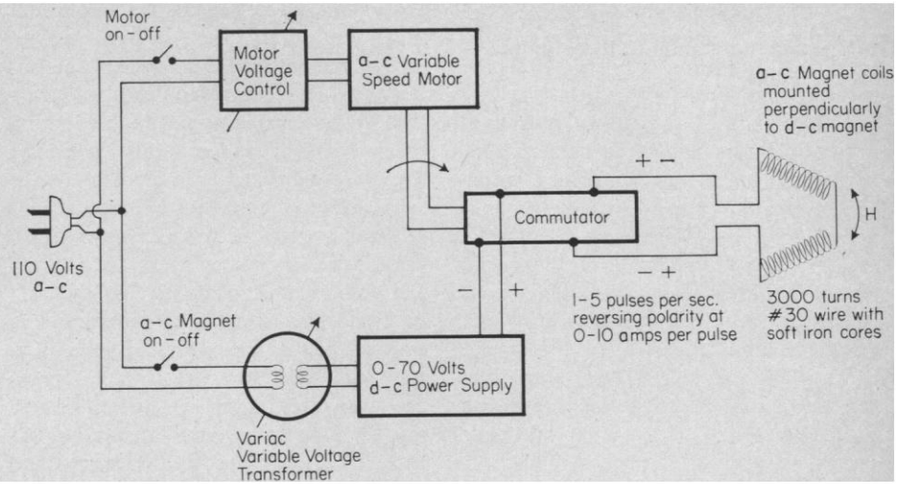


Fig. 2. (Above) A photograph of the external a-c–d-c magnet. (Right) A circuit diagram of the external magnetic control system.

silver-soldered to a coiled stainless steel electrode 0.1 mm in diameter, which is then sheathed in a hollow silicone rubber catheter of the same outer diameter as the magnet. The magnets have the following properties: ${}_BH_c$, 8000 to 9000 oersteds; B_r, 9000 gauss; and a maximum energy product of 16 to 20×10^6 gauss-oersted. This catheter is bonded to the magnet by means of a silicone rubber agent. The resultant catheter resists clotting and is nonreactive to tissue; it has the flexibility to traverse the convolutions of the cerebrovascular system and the strength to function as a carrier for the small magnet and the electrode and to permit safe retrieval (Fig. 1).

The external magnet guidance unit consists of a 5-pound (2.3-kg) permanent horseshoe magnet of Alnico-5 and a dual coil a-c magnet with soft iron cores (Fig. 2). The permanent magnetic field and the a-c magnet field are perpendicular to each other and approximately parallel to the front surface of the guidance unit. Both magnets are embedded in a cast epoxy resin, which locates the magnet poles 1 cm behind the front surface of the epoxy resin. The rear portion of the horseshoe magnet provides a handle for the guidance unit and is also coated with a thin layer of resin. A guiding torque can thus be applied to the magnetic catheter by the permanent field while a simultaneous oscillating torque is applied at right angles to the guiding torque to vibrate the catheter tip without affecting the guidance. The a-c magnet has a variable voltage and frequency. A perpendicular alignment of the a-c and d-c magnets minimizes the demagnetizing effect of the a-c magnet on the Alnico-5 material. Equation 1 shows that the torque applied to the magnetic catheter tip is directly proportional to the magnetic field at the site of the catheter tip:

$$T = mH \sin \theta \qquad (1)$$

T is the turning torque in dyne-cm/cm^3, m is the magnetic moment of the catheter magnet, H is the external magnetic field in gauss, and θ is the angle between the magnetic axis of the catheter magnet and the external magnetic field. For small angles, this equation is approximated by the expression

$$T \sim mH\ \theta \qquad (2)$$

where θ is measured in radians. As a result of this torque, the tip can be turned left, right, or tilted back and forth by similar motions of the guidance unit. The degree of control falls off with increased separation of the magnets. The external d-c field decreased from 400 gauss at 2.0 cm from the front surface of the control unit to 90 gauss at 10 cm. Since the controlling torque also decreases at increased separations of the guidance unit and the catheter magnet, a greater rotation of the guidance unit is required to provide a given amount of turning torque. When the rotation angle required to obtain an effective turning torque approaches 90°, control is lost. The magnetic force exerted on the catheter magnet is proportional to its magnetic moment times the spatial gradient of the ambient magnetic field. The magnetic moment depends on the material and its geometry and on the magnitude of the ambient field. For perfect permanent magnet material, the magnetization is nearly independent of field. Although the magnetomotive force is relatively small with our external unit, the combination of external alternating and direct current and the internal permanent magnet permitted guidance of the catheter tip at distances of 10 to 12 cm. It is not actually necessary for the tip to provide all the motive power; the blood flow and the a-c component of the field are of great assistance. The C-arm fluoroscope with image intensification and video tape capability allows assessment of the control and guidance of the magnetic catheter. The catheter may be visualized not only by its radiopaque tip but by injection of radiopaque contrast material.

The catheter is introduced into either carotid or femoral artery under local anesthesia by standard techniques well known in angiography. The catheter is then mechanically guided to the desired location with magnetic and fluoroscopic control. Entry into arterial branches may require magnetic guidance. Entry into the middle cerebral artery, however, is facilitated by the fact that this artery is the main line of blood flow, as is evidenced by the preponderance of emboli and metastases that occur in its distribution; magnetic guidance, in our opinion, is not usually necessary for catheterization of this vessel.

The control and guidance techniques were first established in a transparent model of the cerebral vascular system constructed to simulate closely the dimensions, curves, and pulsatile flow rates of the major human cerebral arteries. Within the limits of experiments with ten primates (rhesus macaque), this catheter and the control and guidance techniques have been determined to be effective in catheterizing the extracerebral vessels without hazard to the animal.

Further primate studies have shown that radio-frequency heating of the magnetic-tipped electrode catheter can be used to thrombose arteries. After the catheter has been guided to the appropriate part of the artery it is connected to the positive lead of the radio-frequency generator (Radionics model RFG-3A), the ground lead being connected to a hypodermic needle in the region of the catheter tip. Since the electrode has a bared tip and is connected to an electrical generator, the electrical impedance of the tissue will cause the current to flow from the generator into the tissue in accordance with Ohm's law. Measurements have shown that at radio frequencies below 1 megacycle per second the impedance of the tissue is almost entirely resistive. When the electrode current flows through the tissue, heat is generated in the tissue and not in the tip. To ensure maximum safety, the generator power output falls off rapidly as the tissue impedance increases significantly during the lesion formation, thus preventing thermal regeneration; the generator therefore approaches a quasi-constant voltage source of power and is thermally degenerative. Lesion size depends on the thermal characteristics of the target site and, most importantly, on the rate of blood flow and the area of the electrode tip (the larger the tip diameter, the larger the lesion size if the temperature is held constant). A current of 100 ma for 5 to 15 seconds results in a 2- by 3-mm thrombus in the vessel and coagulation of the vessel wall about the catheter tip (*5*). The catheter can be dislodged from the thrombus and successive radio-frequency lesions repeated along the course of the vessel with resultant obliteration of the blood vessel.

We have employed this method to partly obliterate extracranial arteriovenous malformations in two patients whose lesions were of such size and location that they could not be surgically resected. The first case was that of an arteriovenous malformation and fistula of the external carotid system in a 14-year-old girl. This lesion was a rapidly enlarging pulsatile mass in the left parotid region. Apart from the considerable disfigurement of the patient, she complained also of the constant loud bruit. A surgical attempt to reduce the size of this lesion had failed. Examination disclosed a pulsatile, round mass 6 cm in diameter, with a thrill and a bruit and with discoloration of the overlying

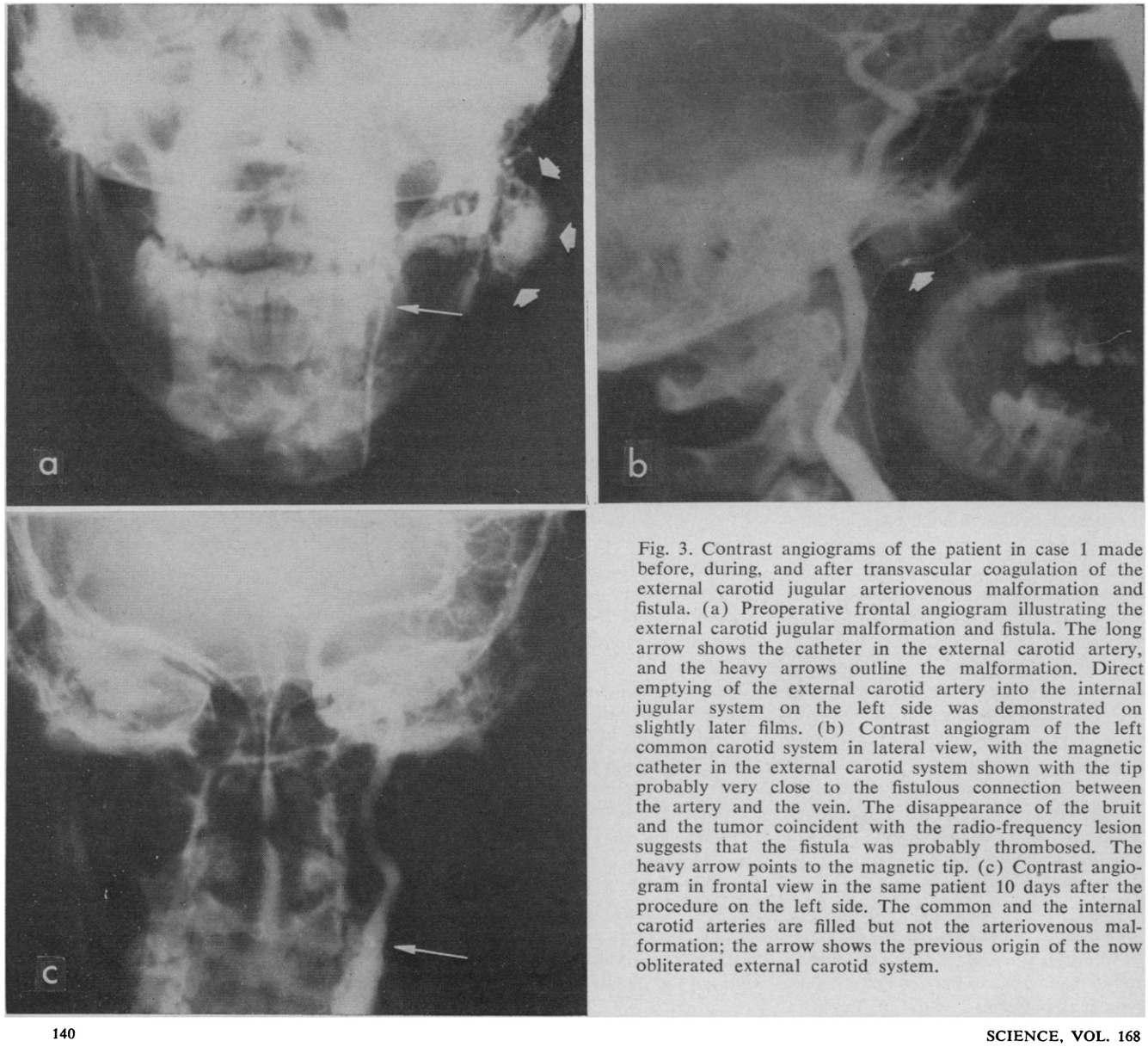


Fig. 3. Contrast angiograms of the patient in case 1 made before, during, and after transvascular coagulation of the external carotid jugular arteriovenous malformation and fistula. (a) Preoperative frontal angiogram illustrating the external carotid jugular malformation and fistula. The long arrow shows the catheter in the external carotid artery, and the heavy arrows outline the malformation. Direct emptying of the external carotid artery into the internal jugular system on the left side was demonstrated on slightly later films. (b) Contrast angiogram of the left common carotid system in lateral view, with the magnetic catheter in the external carotid system shown with the tip probably very close to the fistulous connection between the artery and the vein. The disappearance of the bruit and the tumor coincident with the radio-frequency lesion suggests that the fistula was probably thrombosed. The heavy arrow points to the magnetic tip. (c) Contrast angiogram in frontal view in the same patient 10 days after the procedure on the left side. The common and the internal carotid arteries are filled but not the arteriovenous malformation; the arrow shows the previous origin of the now obliterated external carotid system.

skin in the region of the angle of the jaw. The bruit, thrill, and tumor subsided coincident with the passage of the transvascular electrode into the arteries feeding this malformation from the left external carotid system; the arteries were then obliterated with successive radio-frequency lesions as the catheter was withdrawn (Fig. 3). Postoperative angiography has demonstrated the lesion to be almost completely obliterated, and the patient is cosmetically much improved and able to resume a normal school life. The bruit that was so annoying to her is absent.

A second case of a congenital arteriovenous malformation involving the left side of the face, palate, lip, and jaw in an 8-year-old girl has been treated in similar fashion. No bruit was present, but this lesion was pulsatile and disfiguring and was hazardous to the patient because the teeth and tonsils were almost embedded in a mass of anomalous vessels. This lesion had also been judged to be surgically nonresectable. Partial obliteration of this lesion was obtained by radio-frequency transvascular coagulation without significant cosmetic improvement but with reduction of the arterial components of the malformation. Postoperative angiography confirmed the obliteration of the major feeding vessels from the left external carotid artery but also demonstrated partial filling of the malformation by other vessels from the opposite carotid system. However, the lesion has been so reduced in size that surgical resection can be attempted.

In both of these cases, the catheter was introduced into the external carotid artery and guided to the lesion site under local anesthesia supplemented by intravenous ketamine hydrochloride (*6*). It is well known that total obliteration of these lesions is necessary to prevent recurrence. Thus, although these cases support the feasibility of the use of transvascular radio-frequency thrombosis in humans, they must await long-term evaluation.

Transvascular radio-frequency thrombosis of cranial blood vessels has theoretical potential advantages: it may be used in the awake patient, and no retraction or destruction of the brain is required. There are also potential disadvantages, however: the risk of spasm or occlusion of the parent blood vessel, and extension of the thrombus or embolization from the catheter's tip. The limited results to date are encouraging in two cases of arteriovenous malformations that could not be treated by any other means. This method is currently being explored in the treatment of intracranial arteriovenous malformations and aneurysms.

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Vibriolytic Antibody-Forming Cells: A New Application of the Pfeiffer Phenomenon

Abstract. *Complement-mediated immune lysis was first described by Pfeiffer after observing an immune reaction against* Vibrio cholerae. *Application of this reaction in agar gel, with viable vibro organisms, gave results which were unique compared to the red blood cell or enterobacterial systems. Among these was the lack of a detectable "background," a lengthened latent period and differentiable results among the three major cell wall antigens characteristic of this bacterial group.*

Lysis of *Vibrio cholerae* in vivo by specific antibody and guinea pig complement was first described by Pfeiffer (*1*). The application of this phenomenon of lysis in the presence of complement has been used in both diagnostic serology and basic immunologic research. Standard techniques have been developed for the detection and enumeration of individual lymphoid cells secreting antibody specific for target red blood cells (*2*). These techniques have also been adopted for the detection of antibodies to antigens such as serum proteins, chemical haptens, or bacterial extracts absorbed onto erythrocytes (*3*). The same technique has been modified so that living bacterial cells may be used directly as the indicator target cells (*4*).

Serologic tests based on lysis have been used as sensitive indicators of serum antibody to *V. cholerae* (*5*). However, the nature or quantity of cells involved in formation of such antibody have not been studied (*6*). The plaque procedure described here permits direct detection and enumeration of cells producing specific vibriolytic antibody in vitro and determination of the rise and fall of the cellular immune response to the various cell surface antigens of *V. cholerae*. The three major surface antigens of *V. cholerae* are found separated on the two strains, Ogawa and Inaba. These share a major common antigen (A), but can be distinguished, with absorbed serum, by a type-specific antigen—B in the case of Ogawa and C with Inaba (*7*).

Adult NIH albino A mice, each weighing approximately 25 to 30 g, were immunized by intraperitoneal injection of vaccine of heat-killed *V. cholerae* of either the Ogawa (antigens A and B) or Inaba (antigens A and C) strains. The bacteria were cultured on brain heart infusion agar for 18 hours and harvested by washing with saline. The vaccine was standardized to approximately 5×10^9 organisms per milliliter. The organisms were then killed by heat (60°C for 1 hour).

We developed a direct plaque assay for vibriocidal antibody to enumerate individual plaque-forming units (PFU). Immunized mice were killed, and their spleens were removed immediately. Dispersed cell suspensions were prepared, and 0.1-ml portions, adjusted to contain 1 to 2×10^7 viable nucleated cells, were added to tubes containing 2.0 ml of 0.7 percent melted Bacto agar (Difco), maintained at 48° to 50°C in a water bath. To each tube, 1 mg of DEAE-dextran (*8*), molecular weight 2×10^6, was added as an inhibitor of the anticomplementary activity of the agar (*2*, *3*). A suspension (0.1 ml) of an