The Isolated Monkey Brain: Operative Preparation and Design of Support Systems*

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MULTICELLULAR tissue aggregates and small whole organs have been successfully maintained in isolated viable states for intensive investigation. The pioneer work of Carrel and Lindberg in the 1940's first demonstrated the practicability of supporting these tissues for protracted periods of time via their own intrinsic vasculature, with the aid of artificial circulatory systems. Cerebral tissue, however, until very recently, has been maintained in a separated, living state only as small tissue explants, slices, or single cells; its nutrient support has been derived from the immersing media, but its normal mode of metabolic exchange across the blood-brain barrier has not been possible.

In spite of recent advances in the field of isolated organ preparation and perfusion technique, permitting sophisticated biochemical and physiological measurements under absolute control of environment, the brain has steadfastly resisted all surgical attempts to be prepared and supported as an isolated organ.

This state of affairs has existed for three reasons: 1) the complexity of the intracranial circulation and its innumerable anastomotic relationships; 2) the extreme dependence of brain tissue on an uninterrupted supply of oxygen and glucose; and 3) the surgical difficulty of isolating and removing the brain without seriously compromising the function of the total organism.

Previous attempts to study the metabolism and physiology of the isolated brain have utilized either isolated head preparations or in situ preparations in which the competing cephalic vasculature was eliminated by ligation or differential perfusion pressure techniques. Unfortunately, in none of these biological models does the brain approach an isolated organ state. To produce a truly isolated organ, we have felt it mandatory for the circulation to enter and leave the brain through totally isolated vessels. In addition, we have insisted that all contiguous tissues competing with brain metabolism be ablated. These absolute requirements have necessitated the development of new operative methods and equipment.

In this first report, we describe in detail our operative technique for preparing the isolated monkey brain as well as the extracorporeal perfusion systems (donor and mechanical) that we use to maintain this biological model in a viable state. In a future report we shall present the metabolic and neurophysiological data derived from studies of the isolated monkey brain.

Operative Method

Monkeys were selected as the experimental subjects because previous work had suggested that the anastomotic associations within the extracranial circulation were limited and it was known that sufficient blood could be provided to brain through the posterior (vertebral-basilar) circulation. To reduce the length of surgery, small animals weighing 6 to 8 pounds were used.

Anesthesia was induced and continued throughout the procedure with intravenous Nembutal (25 mg/kg of body weight). Following careful shaving of the entire head, neck, and both groins, the head was placed in a fixation (orbital-oral) unit (Fig. 1) which provided three points of cranial contact including the roof of the mouth and both infraorbital ridges. A femoral artery and vein were cannulated with appropriately sized polyethylene catheters to monitor blood pressure and to provide for fluid and blood replacement. Each animal was wrapped in a

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Thermo-regulated blanket to maintain an even temperature, which was continuously recorded via a rectal thermistor. EKG monitoring was accomplished with suitable limb leads.

Operative Procedure in the Ventrall Position. Initially, the monkey was positioned on its back on a light-weight, adjustable, miniature operating table with the head hyperextended by means of the orbital-oral fixation unit. The skin and subcutaneous tissues were incised in the midline from the manubrium to the angle of the jaw. The trachea was elevated into the wound and divided between large cotton ligatures after the insertion of a wire spiral endotracheal tube.

The following major operative steps were then performed:

1. All the anterior neck muscles including the strap muscles and the muscles immediately associated with the anterior and lateral surfaces of the cervical bodies were completely removed.

2. The severed trachea together with a ligated and divided esophagus were elevated as a single block of tissue, permitting removal of all muscles associated with the base of the skull including the pharyngeal structures, and permitting the ligation and removal of all components of the external carotid arteries and internal jugular veins.

3. The mandibular joint was exposed, and the tissues of the mouth were incised to provide direct communication from the oral cavity to the mandibular joint on each side.

Operative Procedure in the Dorsal Position. The monkey was next rotated onto its stomach and the scalp and tissues of the neck divided in the midline from the nasium to the spinous process of T-1. Six stainless steel paired electrodes were positioned extradurally in small openings in the skull in the frontal, parietal, and occipital areas. They were cemented in place with quick-setting dental acrylic (Fig. 2).

The following major operative steps were then performed:

1. All the tissues and muscles of the skull (temporalis muscles, etc.) and neck muscles associated with the sinus processes and lamina of the upper five cervical vertebrae were totally removed.

2. Both orbits were exenterated, and the fleshy components of the nasal and facial structures were sacrificed.

3. Large bilateral craniectomies were performed, leaving only a bony support for electrodes. In addition, the bone was totally removed from the posterior fossa, exposing the dura covering the cerebellum and lateral sinuses.

4. A generous laminectomy from C-1 through C-5 was accomplished with great care to avoid entering the vertebral venous sinuses (Fig. 3).

Second Operative Procedure in the Ventrall Position. The animal was again rotated onto its back, and following stabilization of arterial pressure, the ventral dissection was completed. The following steps were performed:

1. The lower jaw was removed, with careful control of the volume drainage from the veins associated with the cranial nerves in the base of the skull.

2. After heparinization (3 mg/kg of body weight) the common carotid arteries were cannulated with specially designed silicone coated metal “T” cannulae (Fig. 4). The cannulae were properly located in position by means of “stirrups” constructed from heavy malleable wire.

Second Operative Procedure in the Dorsal Position. The animal was then rotated onto its stomach, its final operative position, and the dorsal dissection and isolation completed. The carotid cannula system was connected to the extracorporeal perfusion system so that the preparation could be instantaneously transferred to extracorporeal perfusion. The isolation of the brain then was carried out as follows:

1. The spinal dura was opened wide in the midline, and with the Ferguson ligature car-
rrier, both vertebral arteries were ligated intradurally just before they joined to form the basilar artery.

2. The cord was doubly ligated at C-1 and C-2 and divided; the anterior spinal artery was divided in the same procedure (Fig. 3). The spinal column between C-1 and C-2 was sectioned.

3. Previously placed ties on the cardiac end of the carotid arteries were closed, the extracorporeal perfusion circuit opened, and the neurovascular bundle divided bilaterally.

4. After satisfactory extracorporeal perfusion had been established and the overhead fixation unit applied to the external auditory canals to provide overhead suspension, the facial, nasal, and upper jaw structures were removed.

Monitoring Techniques

During the operative procedure for isolation of the monkey brain, discontinuous measurements of hematocrit, pH, and arterial oxygen saturation were performed. Small increments (5 to 20 cc) of compatible monkey blood and 6% Dextran were infused to maintain arterial pressure and replace blood loss.

Careful observance of the respiratory condition of the animal was maintained. Initially, the monkey was permitted to breathe spontaneously; however, at the slightest suggestion that the respiratory exchange was inadequate, the endotracheal tube was connected to a Bird respirator and the animal provided with assisted ventilation, using 100% oxygen. To minimize dead space (the estimated tidal volume of the small Rhesus monkey averages only 20 cc) a Bird infant breathing circle was used with the respirator.

Following brain isolation and fixation in the supporting system (Fig. 5), intracerebral temperatures were recorded using a 21-gauge needle thermistor mounted with clay on the support ring and advanced 2 cm into the parietal lobe. In addition, an extradural disc thermistor was placed beneath an appropriate bone edge to estimate the cortical temperature.

Donor Perfusion System

Large, hematologically compatible monkeys weighing from 23 to 41 lbs were selected as donor animals. Each was anesthetized with intravenous Nembutal (24 mg/kg of body weight) and the groin shaved and sterilized. A femoral artery and vein were exposed with great care in order to minimize oozing from exposed tissues during prolonged anticoagulation.

Following heparinization (3 mg/kg of body weight), a right-angle siliconized stainless steel cannula (Fig. 4) was placed in the femoral artery, and a suitably sized plastic catheter was secured in the femoral vein. The animal was positioned in a special chair (Fig. 6, 7) which permitted comfortable restraint in the awake state, as well as rapid changes in the animal's position to support the cardiovascular system during periods of circulatory embarrassment. The femoral arterial cannula in the donor animal was connected with tygon tubing (2 inch diameter) of the shortest possible length to the "isolated brain's" carotid arterial system. For control of incisional pain, the donor's femoral incision was periodically infiltrated with local anesthesia (1% Xylocaine).

At the time of neurogenic, vascular, and osseous separation of the isolated brain, the donor arterial perfusion system (donor femoral artery to the carotid arteries of the isolated brain) was opened, providing uninterrupted arterial circulation to the isolated brain. When the isolated brain had been established on the donor circulatory support system, the venous return from the brain was collected in the transparent, calibrated reservoir of the mechanical perfusion system (Fig. 8), which was positioned beneath the open jugular veins and basilar-vertebral venous sinuses. The cerebral venous blood was pumped (see Fig. 8) from the reservoir through ½ inch diameter tygon tubing to the donor's femoral vein cannula, thereby completing the external vascular circle.

When a "closed" vascular circulation was desired, a rubber finger cot with a small perforation at its tip could be slipped over and ligated about the first cervical vertebral body. A section of ¼ inch diameter tygon tubing, reinforced at its tip with a small cylinder, could then be introduced into the lumen of the cot and ligated in place. This in turn could be connected to the pumping system returning venous blood to the donor.

During the period of isolated brain perfusion, the donor was maintained in an
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Fig. 2. Photograph of the isolated brain during surgical preparation. The osseous EEG electrode system is in place and an extensive craniotomy has been carried out, revealing the dura and brain surface underneath.

Fig. 3. The exposed spinal cord following cervical laminectomy. Two silk ligatures have been passed around it before ligation and division. The posterior spinal artery is easily seen. The posterior fossa craniectomy has not been completed.

Fig. 11. The isolated monkey brain. The brain, suspended in the overhead mounting system, has been on the mechanical support system for 1 hour and 53 minutes. Note the topographical appearance of the brain surface, especially the sulci, gyri, and vessels. The cortical surface electrodes are in place. The arterial perfusion lines are seen feeding into the metal “T” cannulae, which are in place in the carotid arteries.
awake state or under light barbiturate anesthesia. When awake, the animal was fed. If signs of circulatory failure appeared, the body position of the donor was rapidly altered to improve over-all perfusion. Arterial pressure was measured in the brachial (Fig. 6) or femoral artery of the donor after careful cannulation of one of these vessels. Nasal oxygen and external body temperature (heating pads) were easily provided if circumstances dictated their use. To insure a continuous evaluation of the donor’s physiological state during perfusion, discontinuous measurements of blood pH, PO\textsubscript{2}, and PCO\textsubscript{2} as well as arterial and venous oxygen saturation and hematocrits were performed. On occasion, EEG extradural electrodes (bifrontal and biparietal) were placed beneath the donor’s skull through appropriately sized cranial openings (Fig. 6). Cerebral blood flow was measured using time/volume relationships in the calibrated venous reservoir. This reservoir (Fig. 8) also provided an easy vehicle for delivery of drugs (heparin, barbituates, bicarbonates, blood) to the donor.

An even temperature within the extracorporeal system as well as an occasional, rapid, sustained reduction in isolated brain temperature were accomplished by interposing a single hollow-core metal heat exchanger in the arterial line (Fig. 9), which provided absolute temperature control of the arterial blood perfusing the brain. A plastic water jacket was placed around the venous line to rewarm the blood returning to the donor.

At the completion of each experiment, the donor animal was decannulated, given 3 mg of Protamine per kilogram of body weight intravenously to reverse heparinization, and the wounds closed in layers. In addition to local anesthesia (1% Xylocaine) for the surgical closure, the wound was sprayed with a Polynin-Neomycin solution and 600,000 units of Benzothine penicillin administered intramuscularly.

Each donor could provide four vascular sites for perfusion cannulation (two femoral and two carotid arteries) without endangering its immediate survival if sufficient time was allowed before each additional perfusion.

Mechanical Perfusion System

Simultaneously with the separation of the brain from its own circulation, the isolated organ was transferred to a fully mechanized perfusion system (Fig. 10) for uninterrupted maintenance of its circulatory environment. The essential components of this system include: 1) two small pumps, 2) a miniaturized disc oxygenator, 3) a collecting reservoir, and 4) a brain capsule.

![Image](https://via.placeholder.com/150)
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Pump Units. Two small propulsion units were mounted, one on the arterial line and one on the venous line, to actively circulate blood within the extracorporeal circuit (see Fig. 8). Each pump is of an occlusive, eccentric roller-type design with a 7-cm diameter head; compression of a $\frac{3}{4}$ cm diameter rubber boot delivers 0.56 cc per revolution having a pulsatile wave form.

The arterial and venous pumping units operate independently and are capable of a rotational speed of 10 to 180 rpm. Power is drawn from a 24 V standard storage battery, which permits continuous operation for over 24 hours.

Oxygenator. The oxygenating unit is a scaled-down model of the standard rotating disc oxygenator manufactured by the Pemco Corporation (Fig. 10). The gas exchange cylindrical chamber is 18 cm in length and 9 cm in diameter. Twelve circular micro-grooved discs are mounted in the chamber and rotated by a small gear motor attached to the endplate. The base of the gas chamber acts as a reservoir, containing approximately 90 cc of blood from which CO₂ is removed and O₂ added as the blood is filtered and rotated on the discs in a high O₂ tension. A miniaturized Gebauer heat exchanger is placed in the blood chamber, permitting control of circulating blood temperature. This oxygenator was purposely designed for the isolated brain following extensive biological testing to provide ideal oxygenation and minimal destruction of formed blood elements at low flow rates. For example, the blood is 95% saturated; the following combination of variables is used: 50 cc of blood flow, 25 rpm disc rotations, and 2.5 liters of oxygen flow per minute.

Fig. 6. Schematic drawing of the isolated brain donor support system. 1. isolated brain; 2. arterial line; 3. venous line; 4. venous reservoir; 5. venous line motor; 6. control panel; 7. EEG plug system; 8. EEG electrodes in donor animal; 9. pressure transducer catheter in left branchial artery; 10. hydraulic system to rapidly lower or raise the donor.
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with a capsule constructed of commercial Saran Wrap fabricated over a wire skeleton and secured about each metal carotid “T” cannula. The appropriate liquid is introduced into the capsule, completely immersing the brain. The temperature and composition of the bathing solution may be altered as desired. Generally, the brain capsule itself is used to help reduce the brain temperature rapidly by the simple maneuver of filling it with 1°C saline.

Continuous monitoring of the extracorporeal support system is provided by instrumentation that records temperature, pressure, flow, and biochemical alterations within the perfusion media. Temperatures are measured in the collecting reservoir, oxygenator reservoir, on the surface, and deep in the substance of the isolated brain. Perfusion pressures are monitored within the carotid arteries by introducing a small polyethylene catheter directly into the cardiac end of the metal “T” cannula in the carotid artery. All pressure-recording systems used during surgical isolation of the brain and within the supporting equipment during maintenance of the isolated brain employ suitable strain gauge units with continuous multiple readout on a standard Grass recording unit. Likewise, all temperatures are simultaneously recorded on a special Yellow Spring thermo-recording unit providing a permanent temperature as well as continuous visual readout of temperatures.

To provide estimations of cerebral blood flow (CBF) the pumps are calibrated before each run; however, exact measurements of

**Reservoir.** A transparent, calibrated, heat-collecting unit is placed immediately beneath the isolated brain to collect and measure the cerebral venous blood (see Fig. 8). A small-gauge metal screen filter is mounted over the outlet from the reservoir through which blood is withdrawn by the venous pump and returned to the oxygenator. The use of the heating element is optional.

**Brain Capsule.** When control of surface temperature and biochemical environment is required, the isolated brain is surrounded

**Fig. 7.** Restraining chair used for the donor animal during isolated cerebral perfusion. It is inexpensively constructed from thick plywood, two small metal plates, and leather belt restraints.

**Fig. 8.** The collecting reservoir connected to the small vascular motor which is mounted on the venous line in the donor perfusions (See item 5, Fig. 6). This same type of motor is located on both the arterial and venous lines in the mechanical support system.

**Fig. 9.** Examples of three heat exchangers used for perfusion. The two large-diametered heat exchangers are single core units which are used for donor perfusion. The long, thin heat exchanger is multi-cored and used for mechanical support systems.
CBF are easily obtained by recording time-volume accumulations in the venous reservoir. Discontinuous measurements of arterial pH, PO₂ and PCO₂ and venous PO₂ and PCO₂ are made using the Astrup technique. Estimates of blood glucose and lactic acid are likewise determined, to provide continuous data on the biochemical composition of the perfusing media.

Aside from the addition of oxygen and removal of carbon dioxide from the circulating media in the oxygenator, only glucose and sodium bicarbonate are added to maintain the blood glucose above 50 mg/100 cc and to correct the developing acidosis.

Results and Discussion

Sixty-three monkey brains have been isolated and viably maintained on a donor or mechanical support system (see Fig. 11, p. 219). Failure of the preparation during surgical exclusion of the brain from the body has been rare. This has undoubtedly been due to adequate homeostatic maintenance of the animal during surgery; blood replacement, correction of acidosis, control of body temperature, and support of systemic pressure were all important. In addition, because of the small size of the monkeys (6 to 8 lbs), every effort has been made to minimize the loss of blood during surgical isolation. This has necessitated the judicious use of cautery, which in turn has required the immediate and frequent application of cold saline to dissipate the effects of local heat. On occasion, just prior to neurogenic and vascular isolation, the EEG has given evidence of deterioration; however, with institution of the artificial circulation, marked improvement in the cortical electrical activity has been evidenced.

Pilot experiments in the monkey had demonstrated that no neurophysiological deterioration resulted when the brain was perfused only by the posterior (vertebral-basilar) circulation. This information led us to believe that the common carotid arteries could be safely occluded to permit arterial cannulation. The construction of these cannulae allows continued perfusion of the brain via the systemic circulation until transfer of the brain to the new circulation. To close the circle of Willis and thereby distribute blood to all areas of the brain during bilateral internal carotid perfusion, it was necessary to permanently close the basilar-vertebral system. This was accomplished by ligating each vertebral artery under the brain stem close to the formation of the basilar artery. This final vascular arrangement has been commonly referred to as the "closed perfusion circle," thereby perfusing against a pressure gradient enabling circulation to all parts of the brain.
Surgical removal of the nasal structures in the monkey, including the mucosa, is not the major problem in hemostasis that it is in the dog.\textsuperscript{23} This is undoubtedly the result of species variation in blood supply to this area. Exenteration of the orbits can be readily accomplished in the monkey with sparing of the optic nerve for direct stimulation experiments.

Our initial success in isolating the brain in the experimental animal was greatly simplified by the selection of the monkey, whose extracranial circulation is limited in its anastomotic relationships but capable of adequately perfusing the brain via the internal carotid circulation. While it is now possible to surgically isolate the canine brain,\textsuperscript{23} a great deal more effort must be expended in utilizing anastomotic associations within the carotid system, particularly the external carotid arteries, since the internal carotid arteries in this species are incapable of supplying the brain with an adequate blood flow.

Much of our knowledge regarding the metabolic performance of the whole brain has been derived from the “perfused cat brain” preparation of Geiger.\textsuperscript{9} Unfortunately, in this classical \textit{in situ} biological model, no attempt was made to interrupt the basilar-vertebral vasculature. The important contribution made by this posterior arterial system to the feline cerebral circulation has recently been indicated by Wright.\textsuperscript{26} Chute\textsuperscript{8} has demonstrated in his ingenious cat head preparation that 11% of the calculated cerebral metabolic activity was derived from non-cerebral tissue of the head. In our isolated subhuman primate brain all metabolically active tissues have been surgically removed. Since the arterial perfusion goes only to brain and venous return comes only from this same organ, we have, for the first time, a truly isolated brain. The problem of metabolic contamination has thus been eliminated.

Evidence of viability may be maintained in the isolated monkey brain for protracted periods of time with either the donor or mechanical support system. Thus, it has been possible to obtain indications of significant electrical activity and metabolic turnover in the isolated brain for more than 22 hours. During this time, the cerebral sur-

face appears normal with little or no gross evidence of tissue edema (Fig. 11). Arteries and veins coursing in the pia are easily distinguished as are the individual cortical gyri and sulci (Fig. 11). Even after hours of donor perfusion, the isolated brain appears normal when sectioned and examined grossly. Likewise, microscopic inspection of representative sections of brain stained with hematoxylin and eosin appear normal. It should be recalled that, even in the isolated canine brain transplanted in a suitable recipient’s circulation for periods approaching 3 days, cerebral tissue appears normal by light microscopic criteria.\textsuperscript{23}

With interposition of the heat exchanger in the arterial line, rapid and efficient reductions in cerebral temperatures approaching 3°C are possible when the entire brain is immersed in a reservoir containing saline at 0° to 1°C. As a consequence, the metabolic demands of the entire brain can be examined over a wide range of temperature heretofore impossible to achieve via the simple maneuver of simultaneously altering its internal and external thermo-environment.

The donor support system provides the best possible circulatory environment for the isolated brain. Both brains share the same circulation, and since no pump is interposed in the arterial line to the isolated brain, they are both perfused directly by the donor’s cardiac output. Consequently, in the awake donor, the conditions of circulatory support must approach the optimum for the isolated brain.

This advantage of a common circulation is at the same time a disadvantage, since biochemical studies based on arteriovenous differences across brain are limited unless one purposefully alters the arterial inflow composition to the isolated brain. This situation is easily rectified in the mechanical circulatory support system where the perfusate must continuously be presented to the isolated brain, with no opportunity to remove waste products of cerebral metabolism, yet where substances can be easily added to the circulation to study their exact effect on brain alone.

The viable longevity of the isolated brain maintained by a mechanical perfusion system is limited at present. Usually by the third hour of isolated perfusion there is
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beginning evidence of cerebral edema, which is further reflected in the gradual rise of carotid pressure from initial average values of 120 mm Hg to values in excess of 250 mm Hg. This is associated with reduction in electroencephalographic activity and biochemical change. However, even after the electrical activity has become isoelectric and obvious gross cerebral edema has supervened, introduction of Metrazol into the perfusion system will result in an excellent seizure discharge from the cortical surface. On rare occasions, and without explanation, it has been possible to maintain viability in the isolated brain supported by mechanical perfusion for 7 hours.

As a result of the surgical procedure of isolation and methods of circulatory support described, it is now possible to maintain the monkey brain for many hours in a high performance state as a totally isolated organ.

Summary

We have described in detail the operative technique for surgically isolating the subhuman primate brain and the associated methods for maintaining homeostasis and monitoring changes during cerebral exclusion. We have also described in detail the two extracorporeal support systems (donor and mechanical) that maintain the isolated brain, and have indicated their limitations.

References

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