ticipation of an RNA-synthesizing system. The latter alternative seems most likely in sea urchins, (*20*) but whether either of these mechanisms is operative in amphibians is not yet known.

The existence of extranuclear DNA in amphibian eggs has been suggested (*4, 21*). But the synthesis of "DNA-like RNA" does not appear to begin until relatively late in cleavage (*6*), although other classes of RNA may be synthesized shortly after fertilization (*5*). While there has been no clear-cut demonstration of the existence of "stable messenger" in amphibians, results with actinomycin D as an inhibitor of DNA-directed RNA synthesis have strongly suggested that early protein synthesis can occur without the synthesis of RNA (*7*).

Thus, it seems that protein synthesis can occur in the amphibian egg in the absence of continuous nuclear influence. Whether the same proteins are being synthesized in the nucleate and anucleate eggs is not yet known, and the nature of the factors controlling this early protein synthesis remains unclear.

L. DENNIS SMITH
R. E. ECKER
Biological and Medical Research Division, Argonne National Laboratory, Argonne, Illinois 60440

References and Notes

1. R. Briggs, E. Green, T. J. King, *J. Exp. Zool.* **116**, 455 (1951).
2. J. A. Moore, *Exp. Cell Res. Suppl.* **6**, 179 (1958).
3. R. Briggs, J. Signoret, R. R. Humphrey, *Develop. Biol.* **10**, 233 (1964).
4. J. Brachet, *Biochemistry of Development* (Pergamon, New York, 1960).
5. M. Decroly, M. Cape, J. Brachet, *Biochim. Biophys. Acta* **87**, 34 (1964).
6. D. Brown, *J. Exp. Zool.* **157**, 101 (1964).
7. J. Brachet, H. Denis, F. deVitry, *Develop. Biol.* **9**, 398 (1964).
8. H. Tiedemann and H. Tiedemann, *Naturwiss.* **41**, 535 (1954).
9. K. R. Porter, *Biol. Bull.* **77**, 233 (1939).
10. W. Shumway, *Anat. Rec.* **78**, 139 (1940).
11. M. Steinberg, *Carnegie Inst. Wash. Yearbook* **56**, 347 (1957).
12. New England Nuclear Corp., Boston, Mass.
13. Packard Instrument Co., LaGrange, Ill.
14. G. A. Bruno and J. E. Christian, *Anal. Chem.* **33**, 1216 (1961).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
16. L. D. Smith and R. E. Ecker, in preparation.
17. R. E. Ecker and L. D. Smith, in preparation.
18. P. C. Denny and A. Tyler, *Biochem. Biophys. Res. Commun.* **14**, 245 (1964).
19. J. Brachet, A. Ficq, R. Tencer, *Exp. Cell Res.* **32**, 168 (1963).
20. P. R. Gross, *J. Exp. Zool.* **157**, 21 (1964); E. Baltus, J. Quertier, A. Ficq, J. Brachet, *Biochim. Biophys. Acta* **95**, 408 (1965); A. Monroy, R. Maggio, A. M. Rinaldi, *Proc. Nat. Acad. Sci. U.S.* **54**, 107 (1965).
21. E. J. Finamore and E. Volkin, *Exp. Cell Res.* **15**, 405 (1958); J. Brachet and A. Ficq., *ibid.* **38**, 153 (1965).
22. Supported by the AEC. We thank Joan Stachura for technical assistance.

6 August 1965

Brain Transplantation: Prolonged Survival of Brain after Carotid-Jugular Interposition

Abstract. *Six isolated canine brains were successfully transplanted for 6 hours to 2 days to the cervical vasculature of dogs. Viability was shown by electrocortical activity and significant uptakes of oxygen and glucose, with production of carbon dioxide. Cerebral blood flows, temperatures, and pressures of the cerebral homograft were continuously monitored by way of an implantable recording module.*

To extend significantly the viable longevity and improve the biological performance of the isolated brain, permanent vascular implantation in a suitable recipient would be required. Whereas Demikhov (*1*) has demonstrated the feasibility of transplantation of the upper portion of the canine body, including the head, and Guthrie (*2*) has successfully revascularized canine heads for short periods, the more difficult surgical maneuver of transplanting the brain as an isolated organ has not been accomplished. The solution to the inherent biological difficulties of neurogenically and vascularly isolating the brain, upon which cerebral transplantation would depend, has recently been described (*3*). The theoretical possibilities and implications of transfer of cerebral tissue have also been reviewed recently (*4*).

We now describe our experience with transplantation of the isolated canine brain into a recipient dog; we utilized the circulatory environment of the neck. To provide for measuring function during transplantation a survey system has been implanted along with the isolated brain to permit continuous monitoring of the electrocortical activity, cerebral blood flow, temperature, and discontinuous measurement of arterial-venous oxygen A-V_{O_2} and venous-arterial carbon dioxide V-A_{CO_2} and glucose consumption of the isolated brain.

With the dog under sodium pentobarbital anesthesia, the brain was surgically isolated within the skull by meticulous removal of all contiguous tissues. By preserving the integrity of the cranium, support and protection are afforded the brain and its enveloping membranes (*5*). To insure absolute hemostasis during the prolonged period

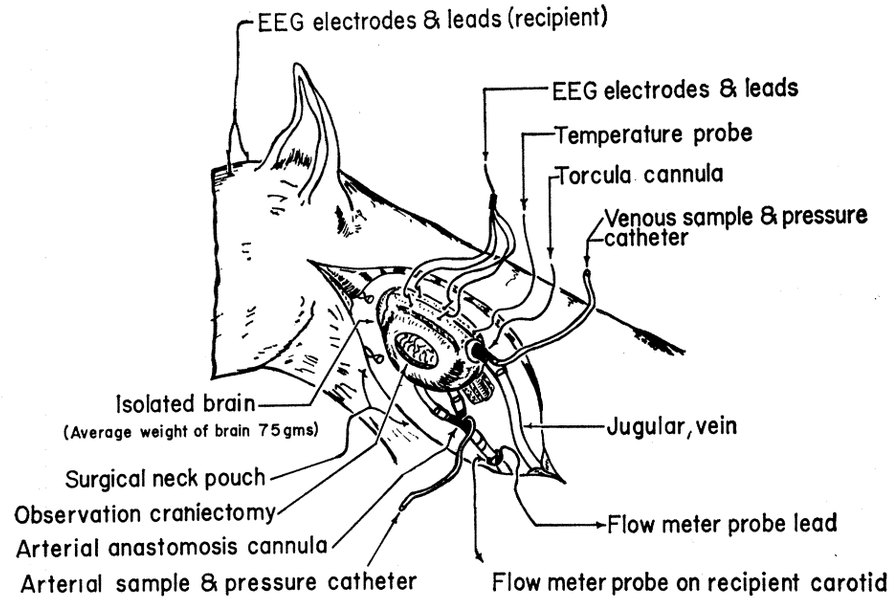

Fig. 1. Isolated brain in position between the carotid artery and jugular vein of the recipient. A small polyethylene tube is connected to the metal side arm of the torcula cannula and provides a means of sampling venous blood from the brain and a means of measuring venous pressure in brain. A similar sampling catheter is connected to the side arm of the arterial cannula. Together with the connections to a 3-mm implantable electromagnetic flow meter probe placed around the recipient carotid artery supplying the brain transplant, the torcular and carotid sampling catheters are brought outside the skin through a small surgical incision. Similarly, the EEG leads and thermoprobe connections are led to the exterior.

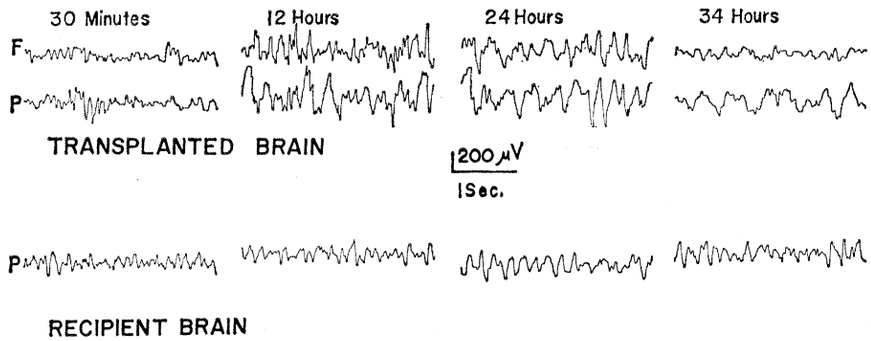

Fig. 2. Spontaneous electrocortical activity from the frontal and parietal areas of the transplanted brain and from the parietal lobe of the recipient brain. The recordings shown were taken ½, 12, 24, and 34 hours after transplantation. At the aforesaid time intervals, brain transplant temperatures were 35°, 33°, 30.6°, and 31.5°C; the recipient's temperatures were 36°, 33.2°, 31.4°, and 31.8°C.

of anticoagulation, thin applications of acrylic cement and gelfoam were applied to the base of the skull and over the frontal bone (denuded of nasal and sinus mucosa) where maximum ooze is encountered. To monitor brain function two pairs of extradural electrodes (for electroencephalography) and one thermoprobe were cemented in the skull over the frontal and parietal areas through small cranial perforations. Cerebral circulation was maintained through the carotid circulation; however, since the internal carotid arteries are incapable of sustaining brain perfusion alone in this species, it was necessary to fashion vascular loops by preserving the anastomotic shunt linking the internal maxillary and internal carotid arteries located in the base of the skull. As the cephalic tissues were removed, so were the various branches of the external carotid artery until only the internal maxillary and the internal carotid arteries and their anastomotic vessels were preserved.

Elimination of the posterior circulation (the anterior spinal artery and vertebral arteries) was accomplished by direct ligation through a wide laminectomy at the second cervical vertebra which also provided ready access to the vertebral sinuses. These were destroyed by means of celloidin replacement injection. Venous return from brain was provided by tapping the torcula after treatment of the animal with heparin (3 mg/kg) with a special cannula which directly shunted the blood into one of the animal's own jugular veins through a Teflon tube. Neurogenic and osteogenic isolation was accomplished by ligating and dividing the spinal cord, also at the second cervical vertebra, and severing the vertebral body at this place. By then the brain was virtually isolated except that it was still perfused by means of its own carotid system. We refer to this preparation as the "autoperfused" isolated brain.

Recipient animals weighing 18 to 23 kg were anesthetized with sodium pentobarbital and intratracheally intubated. After fixation of a pair of extradural EEG electrodes, the neck was surgically opened and the common carotid artery and jugular vein were dissected free along the entire length of the wound on one side of the neck. To provide ample space for the brain, several of the major strap muscles in the cervical area were resected. The isolated brain was removed from autocerebral perfusion by closing and dividing its carotid arteries. The brain graft was weighed, submerged in an antibiotic solution, and transferred to the neck of the recipient animal which had been treated with heparin (3 mg/kg). The two carotid arteries of the isolated brain were connected to the proximal carotid artery of the recipient with a siliconized stainless steel fork cannula. With the establishment of satisfactory outflow from the brain, the torcula cannula was fixed within the jugular vein of the recipient, thus completing the internalization of the circulation of the brain graft.

Since the overall period of circulatory arrest to brain had not exceeded 5 minutes and since intracerebral temperatures at the time of transfer all measured below 31°C, external cooling of brain did not seem necessary. Figure 1 illustrates the cervical location of the brain homotransplant, together with the various components of its implantable survey unit and their connections at the surface of the skin. Prior to surgical closure the wound was irrigated with an antibiotic solution and a small drain was placed in the dependent area of the surgical wound. The recipients were either maintained in a state of light anesthesia or were allowed to awaken. Blood loss that occurred during the anticoagulated state was overcome by replacement with fresh donor blood. At the conclusion of the experiment the cerebral graft was removed, reweighed, and fixed in Formalin.

Six canine brains have been viably grafted to the cervical vasculature of large recipient dogs for periods ranging from 6 hours to 2 days, as indicated by the persistence of significant electrocortical activity. The electroencephalogram of a cerebral graft covering a period of viability approaching 1½ days is reproduced in Fig. 2. The electrocortical activity of the homotransplant can be continuously compared to the tracing of the recipient's brain shown in the lowest channel. Monitoring of the electroencephalogram has been the most sensitive indicator of functional change in the homotransplant. All graft failures have been attributed to low arterial pressure in the recipient or to compromise of intratorcular venous drainage.

Table 1. Average metabolic changes across the transplanted canine brain (av. wt., 75 g). Abbreviations: AP, arterial pressure; CBF, cerebral blood flow; A-V, arterial-venous; V-A, venous-arterial; CMR, cerebral metabolic rate; CRQ, cerebral respiratory rate.

Animals (No.)	Time after transplant (hr)	Brain temp. (°C)	Mean AP, recipient (mm-Hg)	CBF (ml/100 g per min)	$A\text{-}V_{O_2}$ (vol. %)	$V\text{-}A_{CO_2}$ (vol. %)	CMR_{O_2} (ml/100 g per min)	Glucose		CRQ
								A-V (mg/100 g)	CMR (mg/100 g per min)	
4	1	34.8	149	29.1	9.5	8.3	2.8	15.9	4.63	0.87
4	2	34.6	144	19.4	11.6	10.5	2.3	26.6	5.16	0.82
3	4	33.8	149	20.6	10.8	9.7	2.2	39.2	8.08	0.90
3	8	33.7	120	21.8	11.2	11.0	2.5	22.5	4.91	0.98
2	16	32.8	105	30.7	11.8	11.0	3.6	21.3	6.51	0.93
2	24	30.6	92	26.6	11.5	11.0	3.1	13.3	3.51	0.96
1	32	32.2	127	21.3	11.5	10.0	2.5	30.8	6.56	0.87

That transplanted cerebral tissue maintains a high-performance metabolic state for hours after vascular transfer (Table 1) is evidenced by the propensity of brain to protect its intrinsic cellular integrity as it extracts large quantities of oxygen and glucose during periods of reduced cerebral perfusion. Measurements of blood lactates across brain revealed no significant differences between arterial and venous samples. This supports recent work (*6*) suggesting that normal brain does not contribute lactate to cerebral venous blood.

A review of the cerebral blood flows in the homotransplant of the canine brain suggests that previously published values (*7*) for such flow in this species are too high. Certainly our figures for the dog are more in accord with those of McClure and Green (*8*).

Review of histological sections of brain tissue transplanted up to 2 days has failed to reveal any cellular abnormalities (in type or density) which would be compatible with rejection phenomena. Superficial necrosis of the temporal lobes in two brains was probably related to the surgery.

While the present design of the experiment utilizes anticoagulation, the interposition of the canine brain between the carotid–jugular circulation of the recipient "normalizes" the performance of the homotransplant as indicated by the factors reported in this study. Further increase in the longevity of the transplanted, isolated brain and use of the implantable monitoring system should provide unusual opportunities for further brain research.

Our experiments demonstrate for the first time the feasibility of transplanting the canine brain, for protracted periods of time, as an isolated organ into the cervical circulatory environment of a recipient while being able at the same time to monitor continuously its electrical, metabolic, and physiological state.

ROBERT J. WHITE, MAURICE S. ALBIN
GEORGE E. LOCKE
EUGENE DAVIDSON
Neurosurgical Research Laboratories, Cleveland Metropolitan General Hospital, and *Western Reserve University School of Medicine, Cleveland, Ohio 44106*

References and Notes

1. V. P. Demikhov, *Experimental Transplantation of Vital Organs* (Consultants Bureau, New York, 1962), p. 158.
2. C. C. Guthrie, *Blood Vessel Surgery* (Arnold, London, 1912), p. 250.
3. R. J. White, M. S. Albin, J. Verdura, *Science*, **141**, 1060 (1963); *Nature* **202**, 1082 (1964).
4. R. J. White, *Discovery* **26**, No. 3 (1965).
5. The term "isolated brain" refers to the brain contained within the cranial vault, which provides structural support and protection; the two are transplanted as a unit.
6. P. Scheinberg, B. Bourne, D. M. Reinmuth, *Arch. Neurol.* **12**, 246 (1965).
7. K. Sagawa and A. C. Guyton, *Am. J. Physiol.* **200**, 711 (1961); E. Homburger, W. A. Himwich, B. Etsten, G. York, R. Maresca, H. E. Himwich, *ibid.* **147**, 343 (1946).
8. C. McClure and H. D. Green, *ibid.* **197**, 1183 (1959).
9. Supported by PHS research grant (NB 03859–03). We thank R. Yates, J. Austin, W. Nadel, and M. Soloway for technical assistance.

12 August 1965

Efficiency of the Conditioned Eyelid Response

Abstract. *A method for estimating directly the efficiency of the conditioned eyelid response is capable of reflecting marked changes in response efficiency during acquisition of the conditioned response.*

It has been widely assumed that the conditioned eyelid response performs some adaptive function (*1*, *2*). Anticipatory closure of eyelids in response to some conditioned signal may be supposed to afford protection in some degree from the noxious air puff which follows. Nevertheless, this assumption has received scant support from experiments.

Indirect evidence for the adaptive nature of the conditioned eyelid response has chiefly been drawn from measurements of response amplitude and latency. With regard to the first of these it was early shown that the *amplitude* of responding increases during acquisition trials (*3*, *4*), and this has since been confirmed (*1*). It is evident, however, that this increase is not in itself an indication of adaptive change unless the *timing* of the conditioned blink is appropriate to the arrival of the air puff.

It is in this latter aspect that results are conflicting. The most widely quoted recent study (*5*) showed a slight but significant *increase* in response latency (evidenced as a shift in median latency toward the onset of the air puff) during the last 30 trials of a series of 150 trials. Conversely, a *decrease* in latency has been reported over extended training periods (*6*).

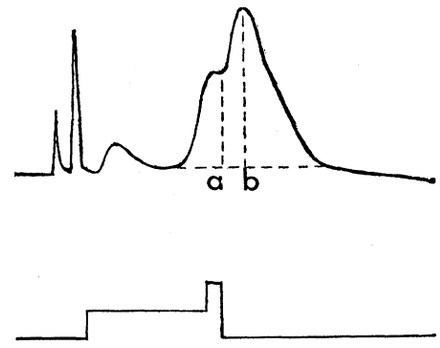

Fig. 1. How the ratio score is measured: *a*, amplitude of CR at point of UCR onset; *b*, maximum UCR amplitude. The ratio is *a*/*b*. The event marker at the bottom shows onset of CS and UCS; the stimuli terminate together.

Campbell and Hilgard also found a decrease in response latency during 50 acquisition trials (*7*). They noted that subjects who could be conditioned most readily had the lowest average response latency. Spence has demonstrated this relationship more precisely by separating acquisition curves for good and poor conditioners, showing that the former decrease while the latter increase in latency over a series of 100 trials (*1*).

While these examples could be multiplied, they suffice to illustrate the contradictory results which arise from the measurement of latency. One possible explanation may lie in the variability of latency, and this is reflected in the fact that it is the least reliable of the standard measures of conditioning—frequency, amplitude, trials to criterion, and latency (*7*, *8*).

In our study, adaptive behavior was assessed by estimating the efficiency with which the conditioned response (CR) enabled the subject to avoid the puff of air; assessment involved selection of an appropriate target latency at which to measure amplitude. Such a target can be functionally defined by measuring the amplitude of the anticipatory CR at the point at which the blink, that is, the unconditioned response, occurs, expressed as a proportion of the amplitude of the reflex blink (see Fig. 1).

We suggest that a convenient definition of the efficacy of the conditioned response is the extent to which it avoids the unconditioned stimulus (UCS) and contributes to or obviates the necessity of the unconditioned response (UCR). Thus a "perfect" CR is