

42 other animals were used as a 2nd donor control group. They did not receive any shock or any special handling. Recipient animals were randomly assigned to receive extracts from 1 of the 3 donor groups. For each group there was a total of 10 recipients, except for the no-escape control group which had 7 recipients. The recipients were tested without reinforcement at 24, 48, and 72-h intervals. Each test consisted of 30 trials with a 30 sec intertrial period in the jump-out box and the number of jump outs were scored. The scores were statistically compared using an analysis of variance with a t-test to see if any group performed better than any of the others. All recipients were injected with the equivalent of 4 donor brains. The recipients were tested under blind procedures. The donor animals were sacrificed by decapitation not more than 2 h after their last training session. The head was immediately placed into a freezer and kept at  $-40^{\circ}\text{C}$  until its entire group was done. The frozen brain was then removed and weighed. The frozen brains were combined with 10 ml of cold ( $+4^{\circ}\text{C}$ ) double distilled deionized water for each g of brain weight, and homogenized (without thawing) using a teflon pestle for approximately 5 min (20 strokes). The homogenate was then placed in a refrigerated centrifuge and centrifuged at  $10,000 \times g$  for 1 h. All operations were carried out under temperatures between 0 and  $4^{\circ}\text{C}$ . After centrifugation the supernatant fluid was poured off and evaporated under vacuum using a flash evaporator at  $0-4^{\circ}\text{C}$ . After the supernatant was entirely dry it was stored in a refrigerator kept at  $2^{\circ}\text{C}$  until it was time to inject and test the recipient animals. Enough cold physiological saline was added to the powder to make the equivalent of 4 donor brains in  $0.5\text{ cm}^3$  of final solution. This was the dose which each recipient animal received i.p. On the 6th day of training of the donor experimental group 10 animals had to be discarded because they had not reached the 90% criteria level.

An analysis of variance was performed on the data. The days interaction was not significant, but the groups interaction was significant ( $F(2,79) = 13.744$ ,  $p < 0.001$ ). A t-test was performed on the combined means for the 3 groups

Group	Mean	df	t	p
Experimental	9.367	79	3.02	$< 0.01$
Shock control	4.571			
Experimental	9.367	79	5.57	$< 0.001$
Control	1.333			
Shock control	4.571	79	2.04	$< 0.05$
Control	1.333			

over the 3 days (table). The experimental group made significantly more jump outs than either the no-escape control or control group:  $t(79) = 3.02$ ,  $p < 0.01$  and  $t(79) = 5.57$ ,  $p < 0.001$ , respectively. There was also a significant difference between the jump out scores of the control and no-escape control group. The no-escape control group made significantly more jumps than the control group,  $t(79) = 2.04$ ,  $p < 0.05$ . Thus, in the testing trials, the recipients injected with the brain extracts of the trained donors exhibited significantly more escapes than both control groups. These results indicate a positive transfer effect. Injections from donors which received only foot shock influenced also the behavior of the recipients, but the number of avoidance responses in this group was in between the responses of mice which received extracts from either naive or fully trained brains.

This experiment indicates that the positive transfer effect is not due to the stress of the trained donors only. If it were so, the transfer effect caused by the extract from trained and no-escape trained brain would be the same.

There are however at least 2 possible explanations of the difference. There might be a quantitative difference in the amount of the active substance in the brain of a trained and a no-escape donor. This causes that the recipients of the no-escape extract escape less often. Another possibility is that we are dealing with 2 different training situations, and therefore with 2 different transfer substances. The no-escape animals learn that they get a painful stimulation in the apparatus; however, they do not learn a proper response, the escape. The recipients react accordingly, but less often than the recipients of an extract of a fully trained brain.

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### Inter omental-cerebral vascularization induced by omental graft to the rat brain<sup>1</sup>

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**Summary.** The omentum of 13 rats were removed from the abdomen and placed directly on the brain. 5-14 days later the omentum and the underlying brain were joined by numerous vascular anastomoses in 9 rats. The purpose of this work was to study the use of omentum to establish extracranial vascularization of the brain.

The omentum stimulates neovascularization when joined to other tissues or organs by trauma, infection or surgery<sup>2-7</sup>. This response has been used by surgeons to channel new supplies of blood to a vascular tissues<sup>3-5</sup>. This is done by opening the abdomen, elongating the omentum, taking care that the vascular attachments to the abdominal arteries are kept intact. The elongated intact omentum is taken out of the abdominal cavity, inserted beneath the skin all the way

to its eventual destination - the leg, brain, arm or wherever. After trepanation, it is placed directly onto the surface of the brain. It has been assumed, that blood flows from the omentum to the brain because omental transposition has prevented cerebral infarction following middle cerebral artery occlusion in dogs<sup>4</sup> and monkeys<sup>5</sup>. Blood flow studies have not been done. Another method has been to remove a piece of omentum, separate it from the abdominal cavity

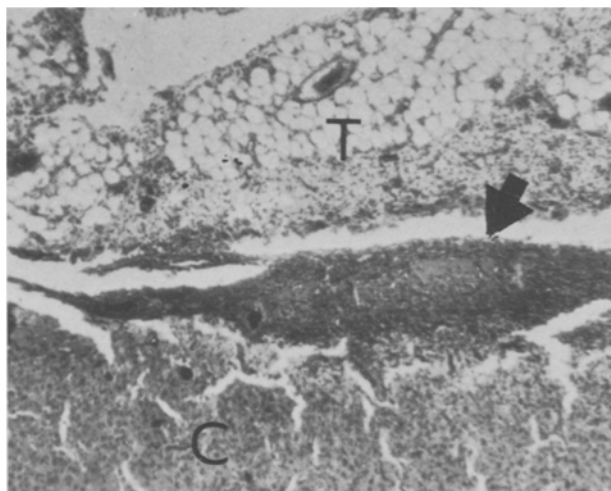


Fig. 1. Site of contact between the omental transplant (T) and cerebrum (C). Note foci of mononuclear cells on surface of cerebrum (arrow).

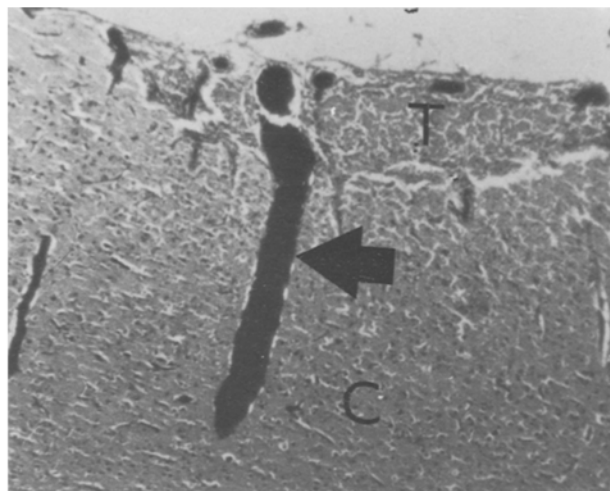


Fig. 2. A portion of transplant (T) on surface of cerebrum (C) shows vessels containing India ink at omental-cerebral interface (arrow).  $\times 100$ .

and place it on the brain where vessels of the omentum and of the brain are joined by microsurgery<sup>6,7</sup>. This method was used to study the possibility of treating hydrocephalus<sup>6</sup>; autotransplantation of omentum was done in a patient with a scalp defect<sup>7</sup>. The purpose of the present study was to find out what happened when a piece of free omentum, without any abdominal attachments, is placed on the surface of the brain. **Material and methods.** Wistar rats were anesthetized with i.p. injection of sodium pentobarbital, 30 mg/kg of b.wt. Following anesthesia, a laparotomy was performed and a 1 cm  $\times$  1 cm piece of omentum was removed and immediately placed in saline soaked sponges to prevent drying. The abdomen was then closed with a running 3-0 silk suture. The rats were then removed from their supine position and placed in a prone position. At this time a mid-sagittal incision was carried down to the underlying periosteum of the skull. This periosteal lining was removed from a portion of the skull and a craniotomy measuring 5 mm  $\times$  7 mm was performed over the temporoparietal area using a dental drill. The dura was found to be so thin in these animals that its inadvertent rupture from the overlying craniotomy caused a cerebral spinal fluid leak from the subarachnoid space. With exposure of the cerebral cortex, the free piece of omentum which had previously been removed at laparotomy was carefully laid upon the underlying brain. This piece of omentum when placed over the craniotomy site not only covered the brain, but overlapped the cranium so that no sutures were necessary to secure the omentum to the underlying brain. After the omentum had been carefully placed, the overlying skin flap was gently laid on the omentum with the skin being approximated using a 3-0 silk running suture. The animals were allowed food and water after awakening from anesthesia.

The experimental animals were divided into 3 groups: a) 5 animals sacrificed 5 days following omental transposition. b) 4 animals sacrificed 10 days following omental transposition. c) 4 animals sacrificed 14 days following omental transposition. Immediately prior to sacrificing the rats, 1 cm<sup>3</sup> of India ink was injected directly into the left cardiac ventricle so that the dye had the opportunity to perfuse the brain. The animals were killed and the brain and its omental attachment were carefully dissected from the skull and placed in 10% formol solution. These tissues were embedded in paraffin, and subsequently cut and stained with hematoxylin-eosin, Holmes silver stain, and hematoxylin-von Gieson solution.

**Results.** The brains of the 13 rats were examined histologically and vascular anastomoses between the scalp, omentum and the underlying brain were found to be present in 3 rats from groups a, b and c respectively, for a total of 9 animals. In 2 rats fibroblasts were at the junction of the omentum and brain, as well as plasma cells, a few phagocytes, in addition to proliferation of capillaries (figure 1). The cerebral tissue at the site of the omental graft in all animals appeared normal, with cortical cells normal in appearance and number, normal myelin and minimal gliosis. There was slight thickening of the dura at the site of the graft, and India ink was present in vessels in the brain, scalp, omental vessels at the site of its adherence to the brain (figure 2).

**Discussion.** These results that vascular anastomoses can be established between the scalp, omentum and brain after a free omental graft is placed directly on the brain tissue. We do not know the direction of the flow of blood between the omentum and the cerebrum and we assume that the blood flows from the area of highest pressure. Thus, if the cerebral vessels were obstructed, the flow of blood would come from the scalp and surface vessels of the brain into the brain. We reported that the intact omentum can prevent cerebral infarction following occlusion of the middle cerebral artery in dogs<sup>4</sup> and monkeys<sup>5</sup>. Intact omental transplantation means the elongation of the omentum, together with its abdominal vascular sources, under the skin to the surface of the brain. Our present studies suggest that free omental tissue can form anastomoses with the brain. This study also shows, as we have seen in the dog and monkey, that there is no destruction of tissue in the underlying brain at the site of the omental transplant.

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