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Cerebral Circulation in the Dog and a Surgical Technique for Isolation of Blood Flow to the Brain

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Introduction

For a number of physiological experiments the cross circulation technique is extremely useful. It is essential however, that complete isolation be achieved. Otherwise, the results obtained are likely to be misleading. Hence, knowledge of the circulation to the brain of the dog is a prerequisite to certain types of study designed to elucidate central nervous chemo-sensitive regulatory mechanisms.

Many attempts have been made in earlier experiments to isolate the head from the body circulation. Except for decapitation experiments, in which the anemia is total, most other ligation experiments permitted long survival times, whether by permanent ligation of both carotid and vertebral arteries[3], or by combined ligation of innominate and left subclavian arteries[11]. These observations suggest extensive collateral flow to vital areas of the brain.

The spinal vessels carry an amount of blood sufficient to mainain the activity of nerve cells in the brain for a surprisingly long period[6,11]. Brown[2] and Hoff[7] also have shown that spinal vessels readily carry dyes to and from the brain.

Others[12] have demonstrated that the main collateral channels are the costocervical and omocervical arteries. Latex injections have shown multiple perforators in the neck muscles beyond the usual point of ligation of carotid and vertbral arteries. The perforators were not open under normal conditions, but in the majority of four-vessel ligation experiments, they promptly open up enough to sustain life. These investigators suggested that the cerebral circulation can be isolated for cross-perfusion purposes by additional ligation of the deep cervical arteries (omocervical and costocervical) at their origins.

More recently, attempts have been made to occlude the collateral circulation in the deep cervical muscles by ligation of the vertebral arteries in the transverse vertabral canal immediately proximal to their point of entry into the spinal canal at the level of C_3[1] or the level of C_2 with pressure cuff around the neck[9] and some workers[8] have ligated the high cervical muscles and soft tissues circumferentially, in addition to intraspinal occlusion of the spinal artery after laminectomy at the C_2 level. Still others[4,10] claim that complete circulatory isolation of the dog's head can be achieved only by combining complete high neck separation of muscles and soft tissues, associated with ligation of vertebral arteries and the deep cervical arterial trunks at the base of the neck.

The original plan was to investigate the ventilatory effect of cerebral arterial perfusion rate, when constant values of PCO_2, PO_2 and PH are maintained in the arterial blood. This preparation requires intact respiratory neural pathways in the recipient animal but with the circulation to its head completely isolated from it's own body and supplied solely by a donor animal.

In the present experiments, several of the recommended techniques were employed; however, careful observations invariably revealed incompleteness of circulatory isolation of the recipient animal's head. This led to a detailed study of the cerebral circulation of the dog and the development of a technique that insured circulatory isolation.

Complete isolation could be achieved by combining complete neck muscle and soft tissue division with major vessel ligation at the level of C_3, only if the vertebral arteries were also occluded in their bony

course immediately prior to entry into the spinal canal. This eliminated the widespread arterial anastomoses mentioned above.

This paper presents the anatomy of the circulation to the dog's head, the surgical procedures for isolating the head, and physiological evidence demonstrating complete circulatory isolation.

Methods

For the cross circulation experiments two mongrel dogs (12-30 kg), the larger one as donor and the smaller as recipient, were anesthetized with 30 mgm/kg of sodium pentobarbital intravenously, and intermittent injections were given to control the depth of anesthesia during the experiment. A long regular tracheal tube was inserted into the donor's trachea, while a tracheotomy was performed for the recipient with a right-angle metal tracheotomy tube. Intravenous physiological saline solution was administered as required during the procedure. Blood pressure was monitored in the femoral artery. The surgical procedures on the recipient dog were as follows:

Carotid denervation. A longitudinal incision was made in the upper neck bilaterally to expose the carotid bifurcation by blunt dissection. The denervation of the carotid chemoreceptor as well as baroreceptor was obtained by disconnection of Hering's nerve. The bundle including these nerve fibers, the occiptital artery originating at the bifurcation, and surrounding connective tissue were divided between two fine silk ligatures.

Drilling of torcular Herophili. In the dog the torcular Herophili is formed by bony walls into which cerebral venous blood flows, thence draining to the osseous transverse sinuses. A midline incision was made on the back of the head, and muscle was dissected from the posterior protuberance. A dental drill was employed to make a suitable sized hole (5 mm in diameter) into the torcular until brisk venous bleeding was seen. A rubber tip conneted to a fine polyethylene catheter was snugly plugged into the hole. Samples of intrcranial venous blood through this catheter were slowly withdrawn to avoid possible contamination with the extracerebral venous blood by retrograde flow from the transverse sinuses

Circumferential high neck division. The skin was incised around the neck at the C_3 level and each bleeding point was ligated. The jugular veins, carotid arteries and vagi were freed and covered with wet sponges of warm saline solution. The entire neck musculature was divided between ligatures in small bundles. In addition all tissues in the front of the neck including trachea, oesophagus and sympathetic nerves were separated in the same manner. Thus the spinal column of C_3 was completely exposed circumferentially without any bridging of soft tissues.

Ligation of vessels at the base of the neck. In low vertebral ligation experiments, the supraclavicular region was opened through a longitudinal incision on both sides at the base of the neck. Using blunt dissection the subclavian, vertebral, costocervical, omocervical and internal mammary arteries were identified and various combinations were ligated. Care was taken not to injure pleura, phrenic nerve, vagi and venous branches.

Vertebral ligation at C_3. For high vertebral ligation experiments, the bilateral transverse processes of C_3, already exposed, were carefully rongeured until the vessels were revealed in the vertebral canal. The vertebral venous drainage was occluded by tiny cotton pledgets above and below. Care was taken to avoid air emboli at this point of the procedure. The well-exposed vertebral artery was then ligated.

Cannulation for cross-circulation. Intravenous heparin 2 mgm/Kg, was given to both dogs ten minutes prior to cannulation. The circulatory connections between the two dogs were made consecutively, alternating venous and arterial channels so as not to disturb the recipient's cerebral circulation. The arterial blood was carried by polyethylene tube from the donor's femoral artery to both vertebral arteries of the recipient at the base of the neck. Prior to cannulation the vertebral arteries were ligated at their origins and common carotid arteries were ligated at C_3. For high vertebral ligation, the arterial tube was introduced into one or both carotid arteries of the recipient after ligation of the vertebral arteries at C_3.

Venous connections from the recipient's jugular veins to the donor's femoral veins were furnished bilaterally.

Vogotomy. in all experiments vagotomy was performed to eliminate the aortic body chemoreflex. The trunk of vagus at the C_3 level was infiltrated with xylocaine and after stabilization of respiration, it was cut bilaterally.

Following the above preparations, the recipient was connected to a spirometer to record ventilation. The following tests were given during experiments.

Evans Blue Test. Evans blue (T-1824, 5 cc of 0.1 % aqueous solution) was injected intravenously into the recipient body during cross perfusion or complete occlusion of the major vessels. Dye detection was obtained by use of the Spectrophotometer from Toruclar samples of recipient 1, 2 and 5 minutes following the injection.

CO_2 Inhalations. 5 % CO_2 in O_2 inspired mixture was given to the recipient only during complete occlusion of the major vessels either with or without cross circulation. The donor dog was air-breathed on a respiratory pump to hold arterial PCO_2, PO_2 and pH constant. On some occasions, arterial donor blood was collected in a constant temperature reservoir prior to pump perfusion.

Latex injection. At the end of the experiments Latex was injected into the aortic arch through a long retrograde femoral arterial catheter. The dead animal was refrigerated for 24 hours before postmortem anatomical examination.

Results

Nineteen experiments are divided into seven groups according to the methods of head isolation employed (Table I).

The adequacy of circulatory isolation of the head was evaluated for each type of preparations by means of, 1) survival time following occlusion of the major feeding vessels of the recipient, 2) Latex injection into the aortic arch of the recipient in an attempt to delineate the presence of cerebral colleteral flow from the "isolated" body following major-vessel occlusion, 3) Evans blue injection into the recipient's body and subsequent sampling of cerebral venous blood at the toruclar Herophili to determine if dye gained entry into the cerebral circulation, and 4) the respiratory effect on the recipient of CO_2 inhalation by the recipient during complete occlusion or cross perfusion. Table II

Table I. The Techniques Used in Attempt to Create Circulatory Isolation of Head from the Body

Group	Procedure
A	Circumferential division of neck skin, partial cut of muscle at C_3, and ligation of vertebral arteries at the origin and common carotid arteries
B	Neck skin and muscle intact, ligation of vertebral arteries at the origin, one subclavian artery and common carotid arteries
C	Complete cut of skin, muscle and soft tissues at C_3, and ligation of vertebral arteries at the origin and common carotid arteries
D	Group C procedure plus ligation of both subclavian arteries
E	Group D procedure plus bilateral ligation of deep cervical arteries
F	Complete cut of skin, muscle and soft tissues at C_3 plus ligation of vertebral arteries at C 4-5 and common carotid arteries
G*	Complete cut of skin, muscle and soft tissues at C_3 plus ligation of vertebral arteries at C_3 and common carotid arteries

*The proposed modification for a complete circulatory isolation of the head

Table II. The results of survival time, Evans blue, CO_2 inhalation and Latex tests for the experiments with complete occlusion of cross perfusion and with partial occlusion of cross perfusion

Complete Occlusion						Partial Occlusion					
Exp. #	procedure	Survival Time (min)	Evans blue	CO_2	Latex	Exp. #	Procedure	Survival Time (min)	Evans blue	CO_2	Latex
2	A	Sacrifice (20)				1	A	28			
3	A	Sacrifice (30)	+			7	C	10	+		+
4	B	Sacrifice (60)		+		10	D	55	+	+	
5	C	6	+			12	E	2			+
6	C	2.5	+		+	13	E	1			+
8	C	Sacrifice (50)	+	+	+	17	G	2		(−)	(−)
9	C	Sacrifice (45)			+	18	G	3			(−)
11	D	Sacrifice (8)			+	19	G	4	(−)		(−)
14	F	Sacrifice (38)	+	+	+						
15	G	0.3			(−)						
16	G	0.5			(−)						

summarizes the results. A positive result is evidence of incompleteness of isolation of the circulation of the head.

Survival time. The most reliable evidence for collateral circulation was prolonged survival time without respiratory disturbance, pupillary dilatation or cardiac failure. No attempt was made to determine quantitatively the collateral flow. Usually, respiratory failure occured first following sufficient reduction or occlusion of the carebral circulation.

In a total of nineteen experiments eleven were able to observe the survival time after complete occlusion and without cross perfusion (Table II, complete occlusion). Seven of eleven were sacrificed after an observed survival time ranging from 8 to 60 minutes. During the period of survival respiration remained essentially normal. Two (#5 and #6) had spontaneous cessation of respiration in 6 minutes and 2.5 minutes. In striking contrast, two others (#15 and #16, both Group G) died rapidly in 20 seconds (3 breathes) and 30 seconds (7 breathes) respectively.

In spite of the high vertebral ligation, dog #14 (Group F) survived for 38 minutes until sacrifice. In this animal a few bundles of deep neck muscle were attached to the spine above the level of the vertebral ligation.

The remaining eight (see Table II, partial occlusion) dogs could not tolerate partial reduction of inflow via the cross-perfusion source. In Groups E and G there were only a few minutes of survival time in five dogs (#12, 13, 17, 18, 19) following diminution of perfusion flow, while dogs #1, 7 and 10 revealed much longer survival times (28, 10 and 55 minutes respectively). Although the rate of reduction of the cerebral flow was different in each of eight experiments, there was generally an inverse relationship between the magnitude of the occlusion and the survival time. Among the various ligation methods only method G achieved total interruption of the shunt channels. Irreversible apnea occurred in less than 1/2 minute when cerebral circulation was not supported by cross circulation.

Evans blue test. The dye detection revealed positive results in all but one experiment (#19, Group G), in which the vertebral artery was ligated at C_3 and the neck muscle and soft tissues were completely divided at the C_3 level.

Because of short survivals after occlusion for all of Group G, only one experiment allowed adequate time for dye-testing. All others died within a short period even if attempts were made to maintain the cerebral circulation by cross perfusion.

CO_2 inhalation. Since the recipient dog's peripheral chemo-receptors were denervated, only leakage from body to head during CO_2 inhalation would produce an effect on ventilation. All experiments revealed hyperventilation during CO_2 inhalation with the exception of #17 in Group G. It

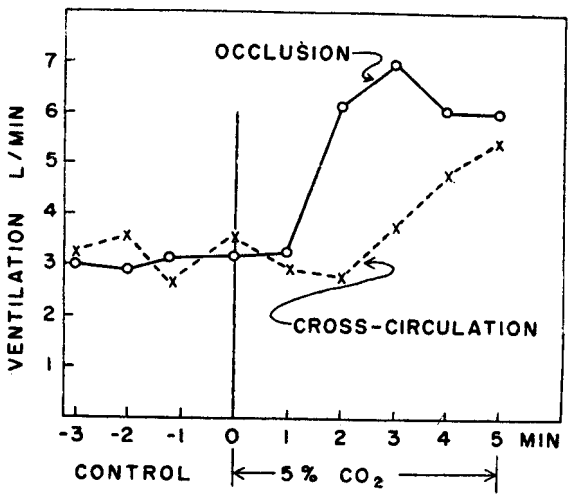

Fig. 1. Hyperventilatory effect by CO_2 inhalation to the recipient dog in which the ceerbral arterial circulation was incompletely separated from the body. Note a rapid CO_2 effect during complete occlusion of the cross circulation due to hypercapnic cerebral blood flow totally from the recipient body (0—0), while a less but still positive CO_2 effect during cross-circulation (×···×), The cerebral blood in the latter is mixture of normocapnic cross perfusion blood and hypercapnic recipient body blood.

was well demonstrated by CO_2 inhalation test in dog #14 (Group F) that cross perfusion did not always supply the entire cerebral circulation of the recipient dog. As seen in Fig. 1, with complete occlusion of major vessels, a rapid CO_2 effect in recipient dog occurred and reached a maximum within three minutes, while a delayed CO_2 effect of lesser magnitude was seen during cross circulation. this effect diminished, but was still positive during cross circulation, and it must be interpreted as due to mixing and dilution of the normally cross-perfused blood with hypercapnic blood contributed from the recipient's body.

Latex injection. Latex was present in the whole intracranial arterial system as well as in the extracranial system including the vertebral arteries and multiple perforators. The Latex in these perforators came from the deep cervical arteries (Fig. 2, Group C). When ligation of the deep cervical arteries (Group E) was performed, Latex found its way into the vertebral artery perforators via muscular branches from the dorsal shoulder muscles. When the subclavian arteries were ligated at the point proximal to the internal mammary artery, Latex gained access to the deep cervical vessels,

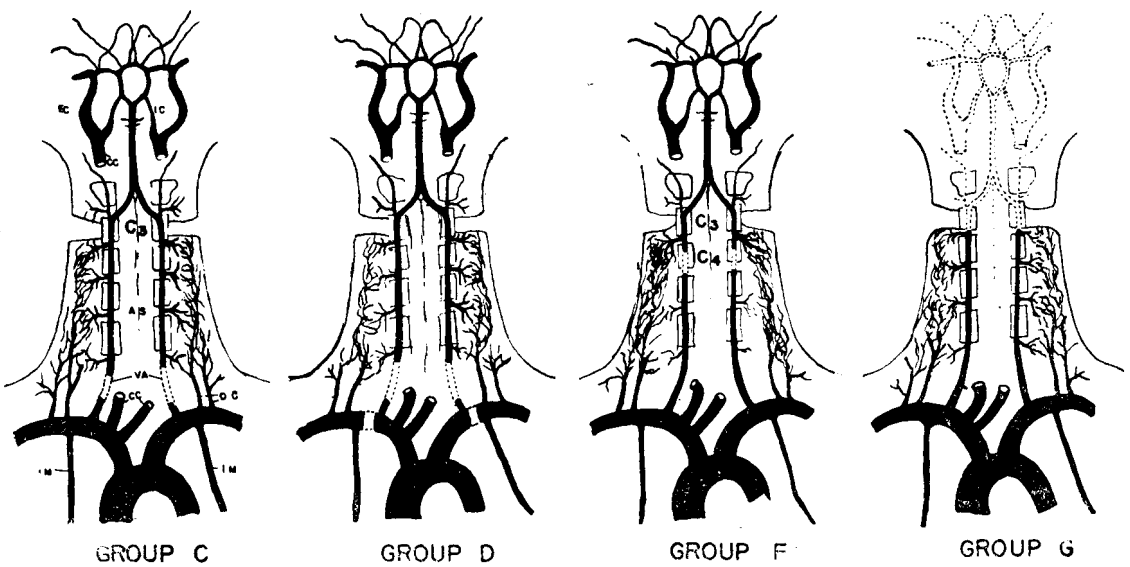

Fig. 2. Schematic drawing of Latex filling in the brain via deep cervical arteries (DC), collateral perforators in the neck, and vertebral artery (VA) following various ligation techniques. Further Latex appeared in the extracranial arterial system beyond the ligation of common carotid artery (CC), No Latex was found in the head of group G.

IM=Internal mammary artery. IC=Internal carotid artery. Ec=External carotid artery. AS=Anterior spinal artery.

and hence the brain by retrograde flow in the internal mammary system (Fig. 2, Group D).

In five animals of group G no Latex appeared in the cerebral arterial system beyond the high vertebral ligation. Also no ascending filling of dye was demonstrable in the small anterior spinal artery in those cases (Fig. 2, Group G). In contrast, Group F showed dye in the cerebral circulatory system via the perforators from cervical muscle to vertebral system between only C_3-C_4 (Fig. 2, Group F).

Discussion

In the dog the vertebral artery runs in the transverse canal up to the level of C_{2-3}, where it divides into three branches. One is the intraspinal portion of the vertebral artery, which enters the spinal canal, ascends to join the contralateral vertebral artery and forms the basilar artery. The other two branches are much smaller; one is the branch ascending into the C_2 transverse foramen, where it communicates with the occipital artery, enters into the spinal canal above C_1 and joins the intraspinal vertebral artery. The others are the perforating branches to deep neck muscles. Multiple perforating vessels from the deep neck muscles connect with the intraosseous portion of the vertebral artery at each segment of the cervical spine. The function of the perforators is not clear in the intact animal, however these undoubtedly open when low vertebral ligation is performed. In many of our experiments the ventilation and systemic blood pressure were not disturbed following gradual occlusion of vessls, probably due to increase of collateral flow. The slight increase of ventilation following each vessel ligation (Fig. 3) was probably due to transient reduction in flow, but it promptly returned to normal. Therefore, vertebral artery ligation should be performed at C_{2-3} or the midportion of C_3, immediately proximal to entry into the spinal canal, in order to exclude these collateral channels. In addition, complete circumferential division of cervical soft tissues at the level of vertebral ligation at the same level is necessary to prevent vascular leakage through skin and muscles. The only remaining artery connecting body to brain is then the anterior spinal artery, which ascends in the midline on the anterior surface of the spinal cord and joins the intraspinal vertebral arteries at their juncture. This vessel is single and very small,—less than 1/2 mm in diameter—and cannot be the main feeding vessel to the brain following major vessel occlusion. There was no filling by Latex in our Group G preparations. Some[2,7,8] have considered the intraspinal portion of vertebral artery as the anterior spinal artery; however, a clear distinction should be drawn here, since the distal sources of blood flow in these two vessels are quite separate. Survival time after total ligation became progressively shorter as the completeness of cerebral circulatory separation was increased. However, a short survival time is not always indicative of total interruption of the collaterals, and other supplementary tests are necessary to evaluate the completeness of separation. In the cross perfusion experiments for studying certain central effects of nervous systems, the collateral shunt channels following complete occlusion of major vessels may lead to confusing results.

The direction of the collateral flow is dependent upon the difference of perfusion pressure from donor and recipien's systemic pressure. If perfusion flow is small or completely clamped and perfusion pressure is less than the recipient's systemic pressure, the cerebral circulation is contaminated or entirely supported by shunt flow from the recipient's body. When perfusion is carried through the carotid system, the brain stem is entirely supplied by the shunt flow. If perfusion flow is high and perfusion pressure is above the recipient's systemic pressure, the perfused blood should not be contaminated by the shunt flow. However, excess perfusion blood leaks out to the recipient body via the perforator channels in reverse direction until both pressures come into balance. This is probably a reason that change of perfusion perssure and flow rate did not alter the vital signs i.e., respiratory pattern, blood pressure, size of pupils or depth of anesthesia in many experiments, in which cases the collateral perforators were well demonstrated. The actual total cerebral flow is

hard to measure when the collateral shunts are widely open.

An effect of CO_2 on ventilation could occur only if there was incomplete interruption of the shunt flow, because the peripheral chemoreceptors (carotid and aortic bodies) were denervated in our preparation. Although Kao (10) did not find any ventilatory change when recipient dogs with intact vagi were given CO_2, the well-known effect of aortic chemoreflex (5), even though it is much less than the carotid chemoreflex, cannot be discounted. Only in Group G was the CO_2 test without effect on ventilation. Hence in this group alone there was no shunt flow.

In seven different types of preparation only the procedure of Group G showed complete separation of cerebral circulation from its own body as determined by prolonged survival time, Evans-blue dye test, CO_2 inhalation test and Latex injection. This new technique provides an adequate experimental preparation for cerebral cross-perfusion studies.

Summary

1) Various combinations of ligation techniques described by others for isolated head circulation were examined to investigate the extent of their completeness.

2) Survival time, Evans blue injection, Latex injection, and CO_2 inhalation by the recipient's body were used to evaluate circulatory isolation of the head.

3) Collateral channels following occlusion of various vessels were demonstrated in the perforators between neck muscle and vertebral system beyond the ligations in most preparations.

4) A modification of complete circulatory head isolation, which excludes total collateral circulation at C_3 in neck muscle and vertebral system, is presented for cross perfusion studies designed to elucidate certain types of central neural chemoreflex mechanisms.

國文抄錄

犬의 腦血液循環과 그의 完全分離法

金 正 根

交叉循環法은 어느 特定한 局所器官에 對한 生理的 또는 藥理的 作用을 硏究함에 있어서 많이 使用되는 法의 하나이다. 特히 腦中樞神經에 있어서이 方法이 많이 使用되고 있으나, 그 結果에 있어서는 願하는 純粹한 腦中樞作用以外에도 願치 않는 全身末梢作用이 混同된다. 이 理由로서는 腦中樞神經에 對한 血液循環을 全身循環에서 完全히 分離함이 他硏究者들에 依하여 使用되고 있는 交叉循環法으로서는 成功치 못하여, 受血者腦의 循環이 供給者血과 不完全分離로 因하여 受血者自身의 副枝血行을 通하는 自身의 循環의 供給과 이를 通하여 供給者血이 受血者全身에 漏出되는 까닭이다,

交叉法에 依한 腦中樞神經에 對한 完全循環分離가 여러 基礎 및 臨床實驗에 있어서 絕對必要하므로써, 이에 他硏究者들로서 考案되었든 이 分離法을 再檢討하여, 그의 不完全性을 生理學的 또는 藥理學的으로 指摘하여 受血者血과 供給者血의 混合을 明示하고, 그 混合經路가 四大動脈인 頸動脈과 脊椎動脈外에 脊椎動脈結紮上部에서 頸部筋肉과 連結되는 副枝血行에 있음을 確認하며 이에 著者의 新完全分離法인 第3頸椎位置에서의 脊椎動脈 및 頸動脈結紮 및 同位에서의 頸筋切斷을 提示하므로써 將來의 腦中樞神經 만의 特殊한 實驗法에 使用될 交叉循環法으로서 提供한다,

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