Rapid Serial Angiography in the Investigation of
the Pharmacology of Brain Circulation in the Dog

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The important contribution of the study of the cerebral circulation to the physiology and pharmacology of the central nervous system has led to the elaboration of new methods for a more extensive investigation of the reactions of the intracranial circulatory area. Each technique has certain advantages and limitations: the direct methods, hemo-
dynamic in principle, allow a continuous reg-
istration of the variations in cerebral circu-
lation, but do not usually provide an absolute
measure of flow; on the other hand, the in-
direct methods, and in particular, the tech-
nique of Kety and Schmidt based on the
Fick principle, provide an absolute measure
but not a continuous registration of the cere-
bral blood flow.

The principal advantage of having both
types of methods available is that the two
are complementary, one providing certain in-
formation not easily obtainable by the other.
In fact, the most convincing data concerning
the physiology and pharmacology of cerebral
circulation are those which support the same
conclusions but are obtained with these two
different methods. In addition, since one of
the principal aims of experimental research
in this field is to provide a comparison with
the results obtained in man, it is evident that
the use, in the experimental animal, of tech-
niques devised for the clinical study of cere-
bral circulation facilitates this comparison.

The application, in the study of cerebral
circulation, of the serio-angiographic tech-
nique, which has been utilized in the study
of other vascular areas, has allowed the deter-
mination of cerebral circulation times in man. Tönnis and Schiele obtained com-
parable results using this method and the one
of Kety and Schmidt.

In our previous work, the cerebral venous
pressure of the dog has been taken as an indi-
cation of the circulatory condition of the
brain, since it is a relatively simple method,
can be carried out with a minimum of trauma,
and provides for a continuous registration
of alterations in circulation. To provide a
further evaluation of this technique, in the
present study, the reactions of the cerebral
circulation to pharmacological agents were
examined in parallel by rapid serio-angiog-
raphy and by the registration of the cere-
bral venous pressure.

Methods
The experiments were carried out on dogs
weighing 12 to 25 Kg. under chloralose anesthe-
(80 to 200 mg./Kg., I.V.) : in the preliminary
observations, the animals were allowed to breathe
spontaneously; in the procedure finally adopted,
the dogs were treated with gallamine (4 mg./Kg.,
I.V.) and were maintained under artificial res-
piration at a rate of 30 to 35 minutes with the
amplitude set in such a manner as to maintain
the arterial and the intracranial venous pressures
at the same levels existing before the administra-
tion of gallamine. This procedure enabled changes
in cerebral vascular tone depending on variations
of gas content of arterial blood to be held to a
minimum.

ANGIOGRAPHIC METHOD
In a short series of preliminary investigations,
the contrast medium was injected into the vertebral
artery through a polyvinyl catheter introduced
into that artery at a point just before it enters
its vertebral canal. In the final experiments, the
carotid route was used: a thin catheter (inner
diameter, 0.5 mm.) was introduced into the common
carotid artery through the superior thyroid artery;
special care was taken to prevent the tip of the
catheter from going beyond the origin of the
internal carotid artery. Regarding the dose of the
contrast medium, a good visualization of the intra-
cranial circulation was obtained by injecting 4 ml.
of compounds commonly employed in the clinical
investigation (sodium acetrizoate, 45 per cent;
Systemic and cerebral vascular reactions to the intracarotid injection of 4 ml. of sodium diace-trizate (50 per cent) in the chloralosed dog. Male
dog: 25 Kg. (10/26/59), chloralose (100 mg./Kg. I.V.). (A) Femoral arterial pressure (mm. Hg); (V) intracranial venous pressure (cm. H$_2$O).
The serigraphic record was taken in the period indicated by the shaded column.

Sodium diace-trizate (50 per cent); the injection was performed under manual pressure as soon as possible; usually the whole dose was injected in about one second.

The sero-angiographic photos were taken in lateral projection by means of the Sanchez-Perez serigraph with the following parameters: intensity 200 ma., 58 to 60 kv.; exposure 0.08 second; focus film distance 100 cm. Since the intracranial circulation time of the dog is rather brief under normal conditions, the complete course of the contrast medium along the cerebral vascular tree could be demonstrated in a series of 8 angiograms taken at intervals of 0.5 to 0.8 second; series of 12 angiograms were only recorded after the administration of drugs which caused marked lengthening of the cerebral circulation time. The sero-angiographic record was initiated at the moment corresponding to the beginning of the injection of the contrast medium. In this way, in the first angiogram it was possible to visualize the internal and the external carotid arteries.

The cerebral circulation time was calculated in two ways: first, by evaluating the time lapse between the beginning of the visualization of the internal carotid artery and of the visualization of the ascending cerebral veins (initial circulation time); secondly, by calculating the interval between the end of visualization of both vessels (final circulation time). The beginning and the end of visualization of these vessels do not always correspond to a definite angiogram; in these cases, following the principles already applied in the clinical investigation, these times have been fixed at the middle of the interval between two consecutive angiograms.

**HEMODYNAMIC METHODS**

The systemic and the cerebral circulatory conditions were followed by registering the arterial pressure from the femoral artery by means of a mercury manometer and the intracranial venous pressure by means of a water manometer connected with a polyvinyl catheter introduced into the external jugular vein and manipulated, by way of the internal maxillary, until the tip was as close as possible to the superior cerebral vein. A continuous tracing of the arterial pressure was taken photographically, whereas the values of the intracranial venous pressure were read every 10 seconds.

**DRUGS**

Strychnine (0.5 mg./Kg.) and ergotamine tartrate (10 /xg./Kg.), which exert clear cerebral vascular effects, were administered intravenously; the motor component of the strychnine convulsions did not appear since the animals were always treated with gallamine (4 mg./Kg.) and maintained under artificial respiration.

**Results**

**PRELIMINARY OBSERVATIONS**

The aim of the first experiments was to establish the most suitable conditions for satisfactory visualization of the intracranial vessels. It was thus observed that the administration of the contrast medium by way of the vertebral artery always provoked marked syncopal phenomena which very often resulted in the death of the animal. Therefore, the carotid route was adopted. Although the injection of the contrast medium by this route often provoked a marked arterial hypotension, due to a transient cardiac arrest, followed by an increase in the intracranial venous pressure (fig. 1), it was observed that the administration of either atropine (0.5 mg./Kg., I.V.) or high doses of gallamine (4 mg./Kg., I.V., fig. 2) completely prevented the appearance of this bradycardia and of the subsequent hypo-
CEREBRAL ANGIOGRAPHY

Absence of vascular reactions to the intracarotid injection of 4 ml. of sodium diacetrizoate (50 per cent) in the chloralosed dog treated with gallamine. Female dog: 12 Kg. (10/30/59), chloralose (100 mg./Kg., I.V.), gallamine (4 mg./Kg., I.V.), artificial respiration. Recordings as in figure 1.

Figure 2

Arterial phase of the carotid angiography (lateral projection): (a) common carotid artery; (b) external carotid artery; (c) internal maxillary artery with its terminal branch ('c'); (d) internal carotid artery with its intrapetrosal (d') and intracranial portion (d''); (e) occipital artery; (f) lingual artery; (g) external maxillary artery; (h) great auricular artery with its terminal branches (h'); (i) superficial temporal artery with its zygomatico-orbital branch (i'); (j) inferior alveolar artery; (m) middle meningeal artery with its branch anastomosing with the internal carotid (m'); (n) ophthalmic artery with its branch anastomosing with the internal carotid (n'); (o) anterior cerebral artery; (p) middle cerebral artery; (O) = catheter.

MORPHOLOGY OF THE CEREBRAL CIRCULATION

The angiographic anatomy of the cerebral circulation of the dog has been described recently, and therefore only the morphological data on which the serio-angiographic evaluation of the intracranial circulation time is based will be considered here. The internal carotid artery and the ascending cerebral veins have been chosen as the starting and the end point of cerebral circulation. The first can be recognized easily by its funnel-shaped origin from the common carotid artery and by its course crossing the occipital and the great auricular arteries and directed toward the intense opacity of the basal bones of the skull (fig. 3). The ascending cerebral veins can be...
detected in the parietal region and present an ascending course leading to the superior sagittal sinus (fig. 4). These vessels collect the blood coming only from the intracranial vessels, whereas other veins, such as the cerebrofacialis and the internal maxillary, collect also a significant portion of extracranial blood. The ascending cerebral veins have been chosen as the end point of the intracranial circulation because they were visualized, under good cerebral circulatory conditions, in all but one experiment (dog 48); in this case, the extremely high rate of the brain circulation probably prevented the brief phase of visualization of these vessels to be fixed in any one angiogram of the series. Numerous extracranial arteries were also visualized by the contrast medium. The more important for pharmacological observations are represented by the great auricular, the superficial temporal, the lingual and the inferior alveolar arteries (fig. 3). The visualization of the extracranial veins was much less evident.

**Cerebral Circulation Time in the Anesthetized Animal**

The results of a group of experiments are shown in the table 1, which gives the data obtained: (a) under basal conditions (chloralosed dog, treated with gallamine and under artificial respiration); (b) during the cerebral vasodilatation induced by strychnine (0.5 mg./Kg., I.V.); and (c) during a phase of cerebral vasoconstriction after the administration of ergotamine (10 µg./Kg., I.V.).

A reliable evaluation of the circulation time can be obtained when the results from dogs under basal conditions are compared with those observed in the animal treated with strychnine. In fact, strychnine is able to restore a normal circulatory condition in the brain in which the circulation has been depressed by anesthesia or surgical trauma and is lacking the physiological and pharmacological reactivity which, in the animal as well in man, indicates a normal hemodynamic picture.2, 10

When data obtained with dogs under basal conditions are grouped with those from dogs pretreated with strychnine, the initial circulation time (see Methods) had a mean value of 2.3 ± 0.33 seconds with fiducial limits (P < 0.05) of 1.46 and 3.14 seconds (seven determinations, five animals); the mean value of the final circulation time was 3.2 ± 0.50 with fiducial limits (P < 0.05) of 1.92 and 4.48 seconds (seven determinations five animals). In the same experiments, the intracranial venous pressure had a mean value of 13 ± 2.10 cm. H₂O with fiducial limits (P < 0.05) of 8.21 and 18.99 cm. H₂O (seven determinations, five animals): these limits correspond to the minimal and maximal levels of the intracranial venous pressure obtained in a wide series of observations.7 Since the absolute value of the intracranial venous pres-
TABLE 1

Cerebral Circulation Time in Dogs Anesthetized with Chloralose

<table>
<thead>
<tr>
<th>Dog</th>
<th>Treatment</th>
<th>Arterial pressure (mm. Hg)</th>
<th>Intracranial venous pressure (cm. Hg)</th>
<th>Internal carotid artery (seconds)</th>
<th>Ascending cerebral veins (seconds)</th>
<th>Circulation time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>Ergotamine</td>
<td>130</td>
<td>14.5</td>
<td>0 to 0.8</td>
<td>1.6 to 4.0</td>
<td>1.6 to 3.2</td>
</tr>
<tr>
<td>29</td>
<td>Strychnine</td>
<td>140</td>
<td>12.0</td>
<td>0 to 0.8</td>
<td>2.4 to 4.8</td>
<td>2.4 to 4.0</td>
</tr>
<tr>
<td>41</td>
<td>Ergotamine</td>
<td>145</td>
<td>9.0</td>
<td>0 to 0.8</td>
<td>2.4 to 6.4</td>
<td>2.4 to 5.0</td>
</tr>
<tr>
<td>42</td>
<td>Strychnine</td>
<td>155</td>
<td>22.0</td>
<td>0 to 0.4</td>
<td>2.0 to 5.4</td>
<td>2.0 to 4.0</td>
</tr>
<tr>
<td>43</td>
<td>Ergotamine</td>
<td>155</td>
<td>13.5</td>
<td>0 to 0.8</td>
<td>1.6 to 3.2</td>
<td>1.6 to 2.4</td>
</tr>
<tr>
<td>45</td>
<td>Control</td>
<td>105</td>
<td>9.0</td>
<td>0 to 0.4</td>
<td>2.8 to 5.6</td>
<td>2.8 to 5.2</td>
</tr>
<tr>
<td>46</td>
<td>Ergotamine</td>
<td>150</td>
<td>14.5</td>
<td>0 to 0.4</td>
<td>1.0 to 2.0</td>
<td>1.0 to 1.5</td>
</tr>
</tbody>
</table>

sure seems to be a reliable indication of the level of the cerebral blood flow, the relationship between the intracranial venous pressure and the cerebral circulation time has been analyzed. A statistically significant inverse correlation between the final circulation time and the cerebral venous pressure has been found (fig. 5A); this correlation was not significant when the initial circulation time was considered (fig. 6A).

### ACTION OF ERGOTAMINE

To evaluate the usefulness of the serio-angiographic method in the pharmacological study of brain circulation, the intracranial circulation time has been determined in the animal treated with ergotamine. Previous work had demonstrated that this drug, administered intravenously in a dose of 10 μg./Kg., provokes a long-lasting fall in the intracranial venous pressure, which results from a vasoconstriction of the cerebral vessels. This action is more evident in the dog in which a marked cerebral vasodilatation has been induced previously by strychnine. The experiments reported here indicate that the fall in the intracranial venous pressure was accompanied by a rise of the cerebral circulation time. Considering the complete series of data obtained under basal conditions, after the administration of strychnine and after the injection of ergotamine, the good inverse correlation existing between the values of the intracranial venous pressure and the final circulation time is confirmed (fig. 5B), whereas the poor correlation between the cerebral venous pressure and the initial circulation time is still more evident (fig. 6B).

In addition to a consideration of the dynamic data, it is worth referring to some morphological modifications of the cephalic angiogram induced by ergotamine. In most cases after the administration of ergotamine the diameters of the extracranial arteries (superficial temporal, great auricular, inferior or alveolar artery) were reduced, whereas the intracranial vessels were seen more clearly as if a greater portion of the contrast medium had now been diverted to the intracranial district. The dynamic effects of ergotamine and some of these morphological modifications induced by the drug are shown in figures 7 and 8.

### Discussion

### CEREBRAL CIRCULATION TIME

The observed values of the cerebral circulation time have shown a variability which appears greater than that obtained by Greitz.
Correlation between the observed values of the intracranial venous pressure (abscissa, cm. \(H_2O\)) and those of the final cerebral circulation time (ordinate, seconds): (A) data obtained under basal conditions and after strychnine; (B) pooled data; (\(O\)) dogs under basal conditions; (\(\Delta\)) dogs treated with ergotamine (10 mg/Kg., I.V.); (\(\Box\)) dogs treated with strychnine (0.5 mg/Kg., I.V.). The data obtained in the same dog are connected by lines as follows: (-----) dog 29; (-----) dog 41; (-----) dog 42; (-----) dog 43; (-----) dog 45; (-----) regression line.

in man (4.13 ± 0.13 seconds). In the experiments referred to here, many factors are responsible for this marked variability; the level of the arterial pressure, varying between 100 and 170 mm. Hg, and the size of animals (12 to 25 Kg.) must certainly play a role. However, there is a more important factor to be considered, namely, the level of the cerebral blood flow. This is clearly demonstrated by the significant inverse correlation existing between the cerebral circulation time and the value of the intracranial venous pressure which is higher the greater the cerebral blood flow. The correlation, which is statistically significant in the group of observations under basal condition and after strychnine, as well as when all the data are pooled, is valid only if the values of the final circulation time are considered. Probably the poor correlation existing between the intracranial venous pressure and the initial circulation time is due to the fact that this time, being shorter than the final circulation time, cannot be evaluated with sufficient accuracy on a serigraphic record taken at a rate of one angiogram every 0.5 second. It is thus possible that, under these conditions, the modifications of the initial circulation time overlap the error limits of the serigraphic evaluation.

On the other hand, the clear inverse correlation between the final circulation time and the intracranial venous pressure enables the variability of the angiographic circulation time to be analyzed. According to the observations of other authors and the results of our previous observations, the minimal and the maximal values of the intracranial venous pressure observed under widely differing experimental conditions are, respectively, 5 to 7 and 23 to 27 cm. H\(_2\)O, whereas the levels of 15 to 20 cm. H\(_2\)O indicate a cerebral vascular condition which, from the experimental standpoint, can be considered to approach a physiological state. Therefore, the values of the cerebral circulation time corresponding to the "physiological" levels of intracranial venous pressure appear to be between 2 and 3 seconds, as indicated by the regression line of figure 5 (A and B).

The quick and marked bradycardiac and hypotensive reaction provoked by the injection of the contrast medium clearly differs from the slower and often less intense fall of the arterial pressure observed by other authors. As the method of rapid serial angiography requires a much quicker injection of the contrast medium, a transient local hypertension can be provoked by this procedure at the level of carotid sinus, giving rise to a stimulation of pressoreceptors and to a reflexogenic increase in the tone of the vagal center. This explanation is clearly substan-
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FIGURE 7

Effect of ergotamine on the angiographic cerebral circulation time. Female dog: 11 Kg. (no. 43, 10/31/59), chloralose (100 mg./Kg., I.V.), gallamine (4 mg./Kg., I.V.), artificial respiration. Rapid seri-angiograms taken after administration of strychnine (0.5 mg./Kg., I.V.) and before the injection of ergotamine. Intracranial venous pressure 20 cm. H_2O; rate of seriographic record: 1 angiogram every 0.5 second. (1) Beginning of visualization of internal carotid artery: first angiogram (0 second); (2) end of visualization of internal carotid artery: second angiogram (0.5 second); (3) beginning of visualization of cerebral ascending veins: third angiogram (1.0 second); (4) end of visualization of cerebral ascending veins: fifth angiogram (2.0 seconds); (5) initial circulation time: 1.0 seconds; (6) final circulation time: 1.5 seconds.

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FIGURE 8

Effect of ergotamine on the angiographic cerebral circulation time. Female dog: 14 Kg. (no. 43, 10/31/59), chloralose (100 mg./Kg., I.V.), gallamine (4 mg./Kg., I.V.), artificial respiration. Rapid sero-angiogram taken after the injection of ergotamine (10 µg./Kg., I.V.). Intracranial venous pressure 14.5 cm. H₂O; rate of sero-angiographic record: 1 angiogram every 0.8 second. (1) Beginning of visualization of internal carotid artery: first angiogram (0 second); (2) end of visualization of internal carotid artery: between the first and the second angiogram (0.4 second); (3) beginning of visualization of cerebral ascending veins: third angiogram (1.6 seconds); (4) end of visualization of cerebral ascending veins: between the fourth and the fifth angiogram (2.8 second); (5) initial circulation time: 1.6 seconds; (6) final circulation time: 2.4 seconds.

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tiated by the fact that some parasympatholytic or ganglionic blocking agents (atropine and gallamine in high doses) are able to inhibit the marked vagal cardiac asystole with the resulting arterial hypotension and thereby the increase of the cerebral venous pressure. This latter is dependent on a cerebral vasodilatation, which, according to the observations of Fog,\textsuperscript{14} represents a compensating reaction of the cerebral vessels to the fall in the arterial pressure.

**PHARMACOLOGICAL OBSERVATIONS**

The serio-angiographic data confirm previous results obtained by means of the registration of the intracranial venous pressure and show new aspects of the cephalic vascular reactivity.

Strychnine markedly reduces the cerebral circulation time; this demonstrates, on a serio-angiographic basis, the increase in cerebral blood flow which can be not only dependent upon the simultaneous rise in the arterial pressure, but also upon the cerebral vasodilatation usually obtained with the administration of strychnine. This vasodilatation, according to combined hemodynamic and electroencephalographic observations, appears to be connected with the enhancement of the functional activity of the brain induced by the drug.\textsuperscript{10}

Ergotamine clearly prolongs the angiographic circulation time, thus giving new evidence of the vasoconstrictor action exerted by this drug on the brain circulation of the chloralosed dog having normal or high levels of cerebral blood flow.

Regarding the extracranial circulation, the narrowing of the arteries in this region induced by ergotamine indicates an extracranial vasoconstrictor action in agreement with the clinical observations of Wolff\textsuperscript{15} and of Bovet and Gatti.\textsuperscript{16}

Also the more intensive visualization of the intracranial vessels often observed after ergotamine is strongly indicative of an extracranial vasoconstriction since this is able to induce a diversion of the carotid blood from the extra- to the intracranial vascular area.\textsuperscript{17, 18}

This effect does not negate previous conclusions on the cerebral vasoconstrictor effect of ergotamine; it merely indicates, when circulation time is also taken into account, an increase in concentration of radiopaque material in the intracranial vessels.

On the whole, these results indicate that ergotamine exerts a cephalic vasoconstrictor action which is more effective on the extracranial vessels; as a consequence, under normal conditions, the drug provokes a diversion of the carotid blood to the intracranial circulation and a simultaneous reduction of the cerebral blood flow, as demonstrated by the lengthening of the cerebral circulation time and by the fall in the intracranial venous pressure. On the other hand, it may be suggested that the diversion from the extra- to the intracranial circulation may be involved in the mechanism underlying the disappearance of the cerebral vasoconstrictor response to ergotamine in dogs with low levels of brain circulation. Under these conditions ergotamine, as well as other vasoconstrictor agents,\textsuperscript{7, 9} no longer reduces the cerebral blood flow, whereas the vasoconstrictor response to ergotamine is still present in the extracranial vessels. Lack of cerebral vasoconstrictor effects has also been seen in man under conditions of reduced cerebral blood flow\textsuperscript{10} and in severe hypoxemia associated with metabolic acidosis in diabetic coma.\textsuperscript{20}

The serio-angiographic observations reported here illustrate, therefore, new aspects concerning the lability of the cerebral vasoconstrictor reactions\textsuperscript{2} and show the usefulness of the rapid serio-angiographic method not only in the pharmacological study of brain circulation, but also in the analysis of problems regarding the relationship between the extra- and the intracranial circulation.

**Summary**

The values of cerebral circulation time were determined by means of rapid serial angiography in the chloralosed dog under control conditions, after administration of strychnine and after injection of ergotamine. These re-
results were compared with those obtained by the simultaneous registration of the intracranial venous pressure. Confirming previous observations made by means of this latter technique, the angiographic results showed that strychnine reduces the cerebral circulation time by provoking an increase in cerebral blood flow, whereas ergotamine causes a cerebral vasoconstriction, thereby increasing intracranial circulation time. Moreover, a statistically significant inverse correlation exists between the values of the intracranial pressure and the cerebral circulation time. New evidence of the relationship between the extracranial and the intracranial circulation was obtained by the angiographic observations; these latter data are discussed in relation with the problem of the lability of cerebral vasoconstrictor reactions.

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References


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