Use of "C-antipyrine for estimation of rhesus monkey eye blood flow

Carol R. Kollarits,* Harold Goldman,** Sharon Murphy,** and Frank J. Kollarits*

The 14C-antipyrine content of rhesus monkey ocular and brain tissues was demonstrated to be stable between 10 and 60 seconds following injection of this diffusible compound, permitting the estimation of blood flow by the indicator-fractionation technique. Blood flow values for central nervous system (CNS) gray and white matter, optic nerve, and retina were similar to values previously obtained for primates by other blood flow techniques.

Key words: blood flow, eye, retina, 14C-antipyrine, diffusible indicators, rhesus monkey, optic nerve, brain, choroid.

Diffusible indicators have been used in the study of central nervous system blood flow for several decades,1-6 but blood flow techniques utilizing diffusible substances have only infrequently been applied to the eye.7-10 14C-antipyrine is a diffusible compound that crosses the blood-brain barrier and has been used previously to estimate brain blood flow in humans and experimental animals.5-12 To investigate the usefulness of 14C-antipyrine for estimation of ocular blood flow in rhesus monkeys, the dynamics of 14C-antipyrine uptake from the blood were investigated in both ocular tissues and brain. Ocular and brain blood flow were determined simultaneously, utilizing a modification of Sapirstein's indicator-fractionation technique.5,7,11,12

Methods

Our standard protocol for determining organ blood flow has been described previously.2 Briefly, the quantity of indicator present in an organ is proportional to its blood flow, if the organ has the same extraction coefficient for the indicator as the whole body at some finite time after delivery.13 Therefore, the validity of the method requires demonstration that organ uptake of indicator does not change during a specific time interval, in spite of continuing recirculation. Under such conditions, the ratio between organ uptake and body uptake equals the ratio (flow fraction) between organ...
blood flow and the cardiac output. To demonstrate that these conditions are met in the rhesus monkey eye and brain, indicator uptake by ocular and brain tissue was determined at intervals during the first 60 seconds following injection of the indicator ($^{14}$C-antipyrine) into the circulating blood.

Healthy adult male rhesus monkeys weighing between 3 and 8 kilograms were anesthetized with intramuscular phencyclidine (Sernylan) (1.0 mg. per kilogram). Atropine (0.04 mg. per kilogram) was given intramuscularly prior to intubation with a pediatric endotracheal tube. Oxygen and nitrous oxide were given by a Harvard small animal respirator attached to the endotracheal tube.

The femoral artery and vein were surgically exposed and catheterized with heparinized PE-60 tubing. The venous catheter was premeasured so that its tip would lie in the inferior vena cava just below the right atrium. The arterial catheter was advanced into the dorsal aorta to the level of the diaphragm. The free end of the arterial catheter was connected to a three-way stopcock, through which blood pressure was continuously monitored by a Statham pressure transducer. Through the second opening of the three-way stopcock, arterial blood samples were drawn for blood gas determinations. Respirator rate, stroke volume and tubing dead space were adjusted to maintain arterial pH between 7.35 and 7.45, Pco$_2$ between 80 and 110 mm. Hg.

After arterial blood gas values were determined, 2,000 U. of sodium heparin was administered intravenously at time ($t$) = -5 minutes. At $t = 0$, a solution containing approximately 25 microcuries of $^{14}$C-antipyrine was injected into the inferior vena cava. At $t = +40$ seconds, the animal was killed by the rapid injection of 5 ml. of saturated KCl solution through the venous catheter. The heart stopped within one beat. In additional experiments, killing times of 10, 20, and 60 seconds following $^{14}$C-antipyrine injection were used in a number of animals to determine the $^{14}$C-antipyrine concentration in ocular tissues at these different times.

After death, the eyes were rapidly enucleated and dissected into 10 parts. Postlaminar optic nerve was excised flush with the sclera. Clear cornea was excised in its entirety, with corneal scissors. As much aqueous humor as possible was collected on a filter-paper disc as it escaped from the corneal incision, and by blotting the front surface of the lens with another disc after removal of the iris. The lens was removed with capsule forceps. The vitreous was gently expressed from the globe which was then cut in half with scissors. The retina was peeled away from the underlying choroid. Histological examination showed that the retinal pigment epithelium remained attached to the choroid. The entire ciliary body was freed from the sclera by sharp and blunt dissection. Blunt dissection was also used to remove the full-thickness choroid (from optic nerve to ora serrata) from the underlying sclera. The entire dissection was completed within 5 minutes after the death of the animal.

The 10 specimens were weighed, minced, and placed in counting vials containing 15 ml. of Bray's liquid scintillation solution. The counting vials containing the scintillation solution and tissues were agitated on a mechanical shaker for at least 2 hours. Extraction of $^{14}$C-antipyrine into the scintillation solvent was found to be essentially complete (> 98 per cent) after 1 hour of agitation. The vials were then transferred to a Packard Tricarb beta counter and each vial was counted for 20 minutes. Standard dilutions of the $^{14}$C-antipyrine solutions were counted before and after the tissue counts were performed. An internal standard and a special quench set were used to correct for machine efficiency and quenching.

Counts were also made on 1 second arterial blood samples collected from the arterial catheter from $t = -5$ seconds to the time of cardiac arrest. These values were used to plot an arterial concentration curve for $^{14}$C-antipyrine. The cardiac output was calculated from the initial slope of this curve, as described previously.

After the ocular tissue had been dissected and weighed, the calvarium was unroofed and 23 samples of central nervous system tissue were dissected and handled similarly to the ocular tissues. Central nervous system blood flow in these animals will be discussed in detail elsewhere.

### Table I. Mean blood flow values in four adult male rhesus monkeys killed 40 seconds after vena caval injection of $^{14}$C-antipyrine

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean blood flow (ml/min/gm. ± S.D.)</th>
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<tbody>
<tr>
<td>Iris</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>0.45 ± 0.11</td>
</tr>
<tr>
<td>&quot;Total anterior segment&quot;</td>
<td>0.72 ± 0.18*</td>
</tr>
<tr>
<td>Retina</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>Choroid</td>
<td>0.39 ± 0.28</td>
</tr>
<tr>
<td>Sclera</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>&quot;Total posterior segment&quot;</td>
<td>1.85 ± 0.39*</td>
</tr>
</tbody>
</table>

*Estimated by measuring the total $^{14}$C-antipyrine in the ciliary body, aqueous humor, iris, lens, and cornea and dividing by the combined weight of the ciliary body and iris.

*Estimated by measuring the total $^{14}$C-antipyrine in the vitreous, retina, choroid, and sclera and dividing by the combined weight of the retina and choroid.
Fig. 1. $^{14}$C-antipyrine time study in 26 adult male rhesus monkeys. Numbers on the abscissa represent the time from vena caval injection of $^{14}$C-antipyrine to cardiac arrest induced by injection of saturated KCl. Values on the ordinate show the per cent of the total dose of injected $^{14}$C-antipyrine found in 1 gm. (wet weight) of each tissue. The number of animals used for each average is indicated by n. The T at the top of each bar graph denotes the standard deviation.
Table II. Blood flow to post-laminar optic nerve and central nervous system gray matter and white matter estimated by different techniques

<table>
<thead>
<tr>
<th>Method</th>
<th>Ref.</th>
<th>Subject</th>
<th>Gray matter ml/min/gm. ± S.D.</th>
<th>White matter ml/min/gm. ± S.D.</th>
<th>Optic nerve ml/min/gm. ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁴C-antipyrine fractionation</td>
<td>*</td>
<td>Monkey</td>
<td>0.56 ± 0.15</td>
<td>0.30 ± 0.09</td>
<td>0.29 ± 0.09</td>
</tr>
<tr>
<td>Xenon-133 clearance</td>
<td>13</td>
<td>Man</td>
<td>0.69 ± 0.09</td>
<td>0.19 ± 0.03</td>
<td>—</td>
</tr>
<tr>
<td>¹⁵µm microspheres</td>
<td>14</td>
<td>Monkey</td>
<td>0.65 ± 0.25</td>
<td>0.25 ± 0.05</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Denotes mean blood flow values from 14 adult male rhesus monkeys killed 40 seconds after vena cava injection of ¹⁴C-antipyrine in the present study.

†Calculated from a value of 100 gm./min./100 gm. dry weight and a dry weight-wet weight ratio of 0.20 for post-laminar optic nerve given by the authors.¹⁴

Results

The results of the ¹⁴C-antipyrine time study using 26 adult male rhesus monkeys are presented in Fig. 1. The uptake of ¹⁴C-antipyrine by each tissue did not vary significantly at 10, 20, 40, or 60 seconds following the intravenous injection of ¹⁴C-antipyrine. The average blood flow values, expressed as milliliters per minute per gram of tissue (wet weight), are presented in Table I for four monkeys sacrificed 40 seconds after ¹⁴C-antipyrine injection.

The average cardiac output for all of the monkeys used in these experiments was 647 ml. per minute (± 213 S.D.).

Discussion

The concentration of ¹⁴C-antipyrine in ocular and central nervous system tissues did not change significantly between 10 and 60 seconds following intravenous injection of a 100 µl bolus of ¹⁴C-antipyrine (Fig. 1). Under these conditions, the percentage of the total dose of ¹⁴C-antipyrine in a given tissue should be equivalent to the percentage of the cardiac output perfusing that tissue.⁵,¹¹

We estimated blood flow from the ¹⁴C-antipyrine content of each tissue 40 seconds after injection because of the relatively constant indicator content of most tissues at this time (Fig. 1).

The blood flow values we obtained for central nervous system gray matter (occipital cortex) and white matter (optic chiasm) are very close to values obtained by other authors using other methods (Table II). Optic nerve blood flow (0.29 ± 0.09 ml./min./gm.) in our monkeys was almost identical to optic chiasm blood flow (0.30 ± 0.09 ml./min./gm.), but retinal blood flow (0.28 ± 0.06 ml./min./gm.) was considerably lower than blood flow to the gray matter of the occipital cortex of the same animals (0.56 ± 0.15 ml./min./gm.). This discrepancy suggested that the ¹⁴C-antipyrine might be diffusing from retina into the vitreous during the interval between the animal's death and the mechanical separation of the tissues by dissection.

Diffusion of indicator from the vascular tissues of the posterior segment of the eye can be accounted for by considering the retina and choroid as a single vascular unit.¹⁵ The capillaries of this posterior-segment vascular unit may be assumed to supply not only the retina and choroid, but also the adjacent sclera and vitreous body. On this assumption, a "total posterior segment blood flow" of 1.85 ± 0.39 ml./min./gm. can be estimated by dividing the ¹⁴C-antipyrine content of the retina, choroid, vitreous, and sclera by the combined weight of the retina and choroid. This value represents an average for the retina-choroid vascular unit since all of each tissue (from optic nerve to ora serrata) was included. If it had been possible to estimate blood flow to the posterior pole alone, a considerably higher value might have been obtained since flat preparations with microspheres entrapped in the vasculature show greater numbers of capillaries in the posterior pole than in the periphery.¹⁴ This anatomical differ-
ence in capillary distribution may explain why the higher value of 5.1 ml./min./gm. for "total retinal blood flow" in the cat was obtained with krypton-85, since the beta-probe in each krypton-85 experiment was placed on the sclera over the highly vascular posterior pole.15

If retinal blood flow is estimated without regard to diffusion (i.e., from the 14C-antipyrine content of the retina alone), a blood flow value of 0.028 ml. per minute ± 0.06 S.D. (n = 4) is obtained for the total retina. Similar values for total retinal blood flow have been obtained with labelled microsphere techniques (0.025 and 0.034 gm. per minute).14, 16 Microspheres obviously cannot diffuse into avascular tissues, and so might be expected to provide results similar to 14C-antipyrine if diffusion of the latter indicator is neglected. From the previous discussion, however, it is evident that a large volume of blood traverses the retinal vessels, supplying diffusable substances to both the retina and adjacent living (although avascular) tissue of the vitreous body.

By a similar argument, the 14C-antipyrine content of the avascular tissues of the anterior segment (cornea, aqueous humor, and lens) can be assumed to derive from the vascular iris and ciliary body. If the total 14C-antipyrine content of all these tissues is divided by the combined weight of the iris and ciliary body, "total anterior segment blood flow" is 0.72 ± 0.18 ml./min./gm. (wet weight iris and ciliary body). This value is considerably higher than estimates based on indicator content of iris or ciliary body alone (0.39 ml./min./gm. for iris and 0.45 ml./min./gm. for ciliary body) and compares favorably with the value of 0.56 ml./min./gm. of dog uvea obtained by the nitrous oxide method.16 This value is also similar to our estimate of chorioidal blood flow (0.59 ml./min./gm. choroid) determined by 14C-antipyrine fractionation, although chorioidal blood flow values estimated with microspheres are considerably greater.14, 16, 17

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