Estimation of retinal blood flow in animals

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The applicability of the krypton-85 (Kr) clearance method to the study of retinal blood flow in cats, rabbits, and monkeys is discussed. Kr disappearance curves are found to be the same whether the isotope is injected via the common carotid artery or via the lateral long posterior ciliary artery. Kr disappearance curves are found to be similar whether the radioactivity is monitored with a Geiger-Muller (beta) probe, a solid state (beta) probe, or a scintillation (gamma) probe. Using the initial slope method of analyzing the Kr disappearance curves, the average rate of blood flow to the retina (from choroidal and retinal vessels) is estimated at 800 ml. per 100 Gm. of retinal tissue per minute.

The recent development of experimental methods for measurement of blood flow in a variety of tissues has stimulated investigation of blood flow in the eye. The only methods adapted to the study of ocular blood flow which have yielded data which could be expressed in terms of blood flow per unit volume of tissue are the outgrowths of the work by Kety and Schmidt.\(^1\) They determined the rate of flow of blood per unit volume of brain tissue by measuring the concentration of nitrous oxide in arterial and venous blood from the brain (from the beginning of inhalation of the gas) and employed the equation for the Fick principle as it applies to a single organ.

Pilkerton and co-workers\(^2\) adapted the nitrous oxide method to the study of uveal blood flow in the dog and reported a rate of flow of 56 ml. per 100 Gm. of uvea per minute.

Another method which has been applied to the study of ocular blood flow and one which does not require the collection of blood samples is the krypton-85 (Kr) clearance method.\(^3\) Jones\(^4\) provided the theoretical basis of this method by demonstrating that the rate of exchange of a rapidly diffusible inert gas between blood and tissue was limited by blood flow. He also showed that the time constant of the desaturation of the gas from a tissue could be used as a quantitative measure of blood flow per unit volume of tissue. Kr is particularly well suited to this type of experiment because in addition to being inert, rapidly diffusible, and radioactive, it has a conveniently long physical half-life (11 years) and a short biologic half-life (0.75 minutes).

In its application in this laboratory\(^9\) to the study of ocular blood flow the Kr was injected intra-arterially, and the rate of disappearance of the isotope was mon-
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 routes with a Geiger-Müller (GM) probe placed over the posterior aspect of the globe. The resultant multiexponential decay curve was analyzed graphically and flow rates were calculated from the rate constant of each of the exponentials. An attempt was made on a radioautographic basis to relate the various exponentials to specific perfusion areas. The purpose of this paper is to describe various refinements of the method and to point out some of its current limitations.

Routes of administration of the isotope

In a previous paper routes were described following rapid injection of $^{85}$Kr (dissolved in 0.9 per cent saline) via the carotid artery. In order to minimize the dispersion of the bolus of $^{85}$Kr in transit to the globe an alternate route of injection was employed.

Monkeys and cats were anesthetized with pentobarbital sodium (30 mg. per kilogram). Rabbits were anesthetized with alobarbital (Dial*) with urethane (0.6 ml. per kilogram). The lateral long posterior ciliary artery (LLPCA) was exposed and cannulated with tapered PE10 polyethylene tubing (Fig. 1). A beta probe was applied to the posterior surface of the sclera and the output of the probe led to a digital rate meter.† Approximately 50 /uc of $^{85}$Kr, dissolved in 0.05 ml. of saline, was rapidly injected into the catheter. The counts were then recorded by a high speed printer,‡ and the data plotted on semilogarithmic paper. Except for a higher initial counting rate, the desaturation curves obtained with injection of the isotope via the LLPCA were similar to those obtained with injection via the carotid artery.

In order to determine the change in intravascular pressure associated with this route of injection, the medial long posterior ciliary artery (MLPCA) was cannulated and tubing was connected to a pressure transducer. The pressure rise in the MLPCA associated with the injection of 0.1 ml. of saline into the LLPCA was 10 to 20 mm. Hg and lasted 1 to 2 seconds.

Radiation detectors

When GM probes were used, movements of the eye were a potential problem and resulted in uncertainty as to the position of the probe in relation to the eye from moment to moment and from one experiment to the next. This difficulty was obviated by suturing a solid state probe to the sclera of the posterior aspect of the eye, usually between the LLPCA and the superolateral vortex vein (Fig. 2). It was possible, thenceforth, to be certain that successive curves pertained to the identical anatomical region. Furthermore the experimental animals did not have to be deeply anesthetized, as slight movements of the globe or the head did not interfere with the experiment.

*Chiba Pharmaceutical Co., Summit, N. J.
†Baird Atomic Model 425.
‡Baird Atomic Model 605.
*Type 6224, Eon Corp., Brooklyn, N. Y.
†Oak Ridge Technical Enterprises, Oak Ridge, Tenn.
Fig. 2. Schematic drawing illustrating positions of various radiation detectors in relation to the eye of the experimental animal during \(^{85}\)Kr desaturation experiments. Total thickness of wall of the eye is 1 to 1.5 mm. Diameter of GM tube window is 6 mm. Diameter of solid state probe window is 3 mm. Diameter of window in lead collimator of scintillation probe is \(3/4\) inch; the diameter of the sodium iodide crystal in the scintillation probe is 2 inches. In the cat and monkey retinal and choroidal vessels perfuse the inner and outer layers of the retina, respectively, an avascular zone intervening. In the rabbit the retinal vessels are relatively insignificant.
Fig. 3. Radioautographs of eyes enucleated at various intervals after intra-arterial injection of $^{85}$Kr. Photograph at left depicts appearance of cross section of tissue (without radioactivity) for comparison with radioautographs on the right, which were developed after separation of tissue from film. Exposure times were as follows: tissue without radioactivity, 2 hours; eye enucleated 3 seconds after injection of $^{85}$Kr, 1 hour; 20 seconds after injection, 2 hours; 2 minutes after injection, 5 hours; 12 minutes after injection, 5 hours. All photographs are at the same magnification. (From Friedman, E., Kopald, H. H., and Smith, T. R.: Invest. Ophth. 3: 539, 1964.) (Obj. x2.5, oc. x10.) Method of preparation of radioautographs has been described elsewhere.\(^5\)

The $^{85}$Kr disappearance curves obtained with the solid-state probe in the cases of the cat and monkey were, in general, similar to those obtained with the GM probe applied to the same area. Repeated determinations were more reproducible. The $^{85}$Kr desaturation curves for the rabbit consisted of 4 exponentials when the solid-state probe was used. This contrasts with the 3 exponentials which were often obtained with the GM probe. This discrepancy is probably accounted for by the difference in the methods of positioning of the two types of probes.

Although $^{85}$Kr is primarily a beta emitter, 0.6 per cent of its disintegrations are gamma rays which can be detected externally with a scintillation probe. External monitoring of $^{85}$Kr disappearance has been successfully applied to the study of myocardial, renal, and cerebral blood flow.

After closing the orbital incision, a 3/4 inch collimated scintillation probe* was positioned over the posterior portion of the globe. $^{85}$Kr was then injected by the same method as was used with beta probes and similar disappearance curves were obtained.

It was, of course, not possible with a gamma detector to be certain of the precise source of radioactivity, even with adequate shielding and collimation. Indeed, when the flow of blood to the eye was stopped by increasing the intraocular pressure above the systolic pressure and the $^{85}$Kr injected via the LLPCA or the carotid artery, a low level of radioactivity, obviously from orbital or periorbital tissues, was still detected by the scintillation probe.

Anatomic localization of perfusion areas

It had previously been demonstrated with radioautographs that the initial radioactivity was localized in the retina and choroid and that most of it had disappeared from these areas in 2 minutes (Fig. 3). As there are two separate vascular beds monitored by the system, an effort was made to ascertain whether they could be identified by specific rate constants in the case of the cat. These experiments were inconclusive and further attempts were made in the monkey. The optic nerve of a monkey was exposed at its junction with the globe. After the rate of desaturation of $^{85}$Kr was recorded, the optic nerve was transected, all vessels but the central

* Baird Atomic Model 815CL.
retinal artery and vein being carefully spared. The $^{85}$Kr injection was repeated, and the desaturation curve was found to be unchanged. Under the conditions of this experiment retinal vessel flow could not be specifically identified by any one of the exponentials.

Calculations

As an alternate to graphic analysis of the clearance curves by extrapolating exponentials, the simplifying assumption was made, on a radioautographic basis, that the choroid and retina comprise one tissue compartment which has practically instantaneous diffusion equilibrium, and that a small amount of $^{85}$Kr diffuses into the adjacent avascular layers (sclera and vitreous). The initial slope of the desaturation curve will then provide an estimate of the average blood flow of this one compartment:

$$f = \frac{\lambda \cdot 0.693}{T_{1/2}} \text{ ml. per gram per minute}$$

where $f$ is the average blood flow, $\lambda$ is the tissue:blood partition coefficient (found to be close to unity for choroid and retina), and where $T_{1/2}$ is the duration in minutes for the decrease to one-half of the functional value of a straight line fitted on semilogarithmic paper to the initial part of the desaturation curve (Fig. 4). The derivation of this equation and the theoretical justification of this method of handling $^{85}$Kr clearance data are to be found in the work of Ingvar and Lassen.8

When calculated in this fashion, the average rate of flow of blood in this single compartment in anesthetized monkeys, cats, and rabbits was estimated to be 800 ml. per 100 Gm. per minute (range: 500 to 1100 ml. per 100 Gm. per minute). Flow rates were found to be highest at the posterior pole.

Transit time of a nondiffusible isotope ($^{131}$I-albumin) in comparison with transit time of $^{85}$Kr.

Because of the extremely rapid rates of flow obtained by this method, it was critical to ascertain whether the initial slope represented the desaturation of $^{85}$Kr which had equilibrated with the tissue or whether it might possibly represent the transit of unequilibrated isotope. When $^{131}$I was injected in previous experiments via the carotid artery only a low level of radioactivity was detected. Injection of the isotope via the LLPCA in this series of experiments made possible the estimation of the $^{131}$I-albumin transit time (3 to 4 sec.) (Fig. 5).

Comment

The goal of the present series of experiments is the development of a method for estimating the rate of perfusion of the retina by blood. This has involved a consideration of the potential role of choroidal blood flow as well as retinal vessel flow. The term retinal vessel flow is self ex-
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Fig. 5. Comparison of semilogarithmic plot of $^{131}$I albumin disappearance curve followed by $^{85}$Kr disappearance curve in the same experimental animal. Both isotopes were injected via the LLPCA of the cat and monitored with a CM tube. The $^{131}$I albumin curve is characterized by a single spike with a total duration of 3 to 4 seconds, followed by a relatively low level of radioactivity which decayed slowly. The initial spike was interpreted as the transit time of $^{131}$I albumin. The slowly decaying background was attributed to recirculation or to the persistence of a small amount of $^{131}$I albumin in the tissue.

planetary. In a superficial sense the phrase choroidal blood flow might be defined as the volume of blood circulating in the choroidal blood vessels per unit weight of choroidal tissue per unit time. As the choroid, however, consists largely of blood vessels, and the choriocapillaris perfuses the outer layers of the retina, it would appear to be more meaningful to define choroidal blood flow as the volume of blood circulating in the choroidal blood vessels per unit weight of retinal and choroidal tissue per unit time.

The single compartment, therefore, the rate of perfusion of which has been postulated to be characterized by the rate constant of the initial slope of the $^{85}$Kr desaturation curve, comprises the choroid, retina, and retinal blood vessels. The relative contribution of each of the two circulations (choroidal and retinal) to the observed rate of perfusion has yet to be clarified. The results of earlier experiments suggested that retinal vessel flow (166 ml. per 100 Gm. per minute) could be differentiated from choroidal vessel flow (1,200 ml. per 100 Gm. per minute). Anderson and Saltzman, using their figure for retinal O$_2$ utilization in man (11.5 ml. of O$_2$ per 100 Gm. retinal tissue per minute) and the figure of Hickam and Frayser for the retinal arteriovenous O$_2$ difference in man (7.4 ml. of O$_2$ per 100 ml. of blood) calculated retinal vessel flow to be 135 ml. per 100 Gm. per minute. If one uses the same figure for retinal O$_2$ consumption (11.5 ml. per 100 Gm. of retinal tissue per minute) and the figure of Cohan and Cohan for the uveal arteriovenous O$_2$ difference in the dog (0.9 ml. per 100 ml. of blood), one arrives at a calculated flow rate in the choroidal vessels of 1,280 ml. per 100 Gm. per minute. Trokel, using reflective densitometry in the albino rabbit eye, reported a rate of flow of 7.9 mm.$^3$ of blood per second per square centimeter of fundus. Assuming that the weight of the rabbit choroid and retina per square centimeter of fundus at the posterior pole is 0.05 Gm. (the average...
of four weighings), then the rate of flow of the blood in the choroidal vessels is approximately 950 ml. per 100 Gm. per minute. These flow rates are in remarkable agreement with those calculated from the rate constants arrived at by graphic analysis of \(^{85}\text{Kr}\) clearance curves. Despite this agreement, a compartmental analysis of \(^{85}\text{Kr}\) clearance curves has yet to be justified, and pending additional information the initial slope method appears to be a more tenable method of estimating average retinal blood flow (in choroidal and retinal vessels).

The absence of consistent change in the clearance curves following obliteration of the retinal vessels can be explained by the relative inefficiency of beta probes in detecting radioactivity in distant tissues (1 to 2 mm.) in comparison with those closer to the probe. It is also possible that the mean transit time of \(^{85}\text{Kr}\) circulating through the retinal vessels is too similar to that of \(^{85}\text{Kr}\) in the choroidal circulation to be identified by a unique time constant. Measurements with beta probes upon the surface of the retina might help to distinguish between these possibilities.

Although the initial slope method of analyzing the clearance curves does not enable one to determine the volume of blood flowing through the choroidal and retinal vascular beds individually, one can measure the capacity of these two circulatory systems, in combination, to supply diffusible substances to and remove them from the retina. The high degree of efficiency of these circulatory processes as reflected by the \(^{85}\text{Kr}\) clearance data is of special significance in the case of the retina, which maintains a remarkably high rate of \(O_2\) utilization despite the avascularity of its outer layers.

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