Technical Papers

Rapid Serial Recording of Concentrations in the Blood Circulation and in Perfusion Systems: The Effluogram[1, 2]

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Recently we have attempted to relate in time the outflux of potassium from cardiac muscle to the phases of the contraction myogram and of the action potential. The heart of the intact turtle is pre-equilibrated overnight against intraperitoneal K^{42}. The next morning the coronary artery and a vein of the excised heart are cannulated with polyethylene tubing. Nonradioactive Ringer's solution is perfused through the ventricle, which has been rendered quiescent by Stannius ligature. After the ventricle is cleared, by perfusion, of blood and interstitial K^{42}, etc., it is ready for stimulation by inductorium to demonstrate the possible release of radiopotassium into the outflowing Ringer. Such use of a radioisotope adds marked sensitivity to the analyses, but the changes still seem too fast and too feeble to record with a count rate meter placed over the heart or over an effluent vessel.

Instead, we lead the outflowing Ringer onto a strip of filter paper that moves past the cannula at a fast but known speed. Thus the chemical outflux is caught and stored on the strip for leisurely counting later, just as motion is caught on a motion picture film. The K^{42} may be counted later by moving the strip much more slowly past a count rate meter or by counting numbered segments cut from the paper strip. If it is desirable to record concentration—that is, to relate the K^{42} count to the volume of effluent perfusate that contained it—we add a known concentration of biologically inert indicator radioactivity to the perfusate. We have used tagged iodinated albumin and radio iodide in carrier NaI. The I^{131} count is then an indirect measure of the volume outflow associated with a given K^{42} count. The frequency of sampling is limited only by the magnitude of the radioactivity counts obtainable. Not only are samples frequent and small, but there is no wastage of fluid in the sample tubes while pipetting. Serial concentrations in animal blood from cannulated veins or arteries can be measured with a minimum of bleeding. We have recorded samples every 0.1 sec, representing equivalent volumes of 0.003 ml.

The effluogram shown in Fig. 1 was done with thin filter paper mounted on blotting paper (we now use Eaton-Dikeman filter paper #320, which is about 2.5 mm thick). The strip was 1.6 cm wide and 51 cm long. A band of Scotch tape was laid along the bottom and folded over the edges of the strip to make it watertight except for the upper surface. The strip was mounted snugly on a horizontal kymograph drum which moved the strip past the effluent cannula at a rate of 4.56 cm/sec. The myocardium was stimulated by inductorium at a given position on the revolving drum so that outflow from but one contraction was collected along the length of the paper strip. However, outflows from 25 contractions were superimposed by repeated revolutions and contractions. The strip was then dried and cut into numbered segments of fixed length, which were placed into planchettes for counting, using the automatic sample changer equipment of Tracerlab, Inc.

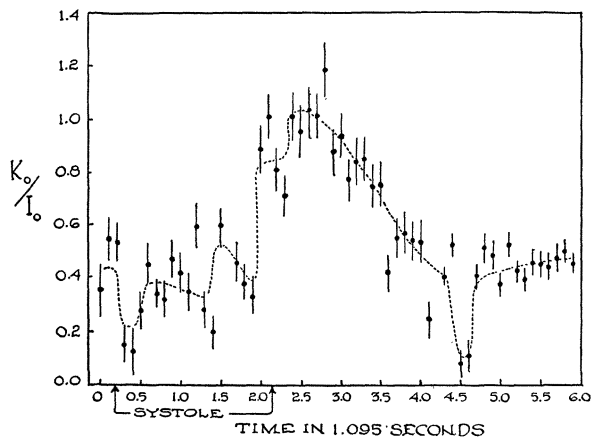

FIG. 1. Effluoradiogram for turtle ventricle pre-equilibrated against K^{42} *in vivo* and then perfused with K^{42}-free Ringer's containing I^{131} as NaI.

As mentioned above, the volume of effluent perfusate caught on each segment is computed from I^{131} mixed into the turtle Ringer ahead of time. The I^{131} and K^{42} counts may be separated partially with a filter, but we usually separate them by counting the two radioactivities together before and after decay of the short-lived K^{42}. If absolute calibration for volume is required, a known volume of perfusate containing I^{131} may be measured onto a paper segment. Self-absorption by the paper is minimal for the energetic β-emission of K^{42}, and a correction factor may be used for the self-absorption of I^{131} β-emission, provided the paper segments are always dried to constant water content.

An effluoradiogram for the turtle ventricle is shown in Fig. 1. The ordinate K_0/I_0 is the ratio of K^{42} to I^{131} counts calculated to be present in a paper seg-

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[2] Isotopes allocated by the AEC. Authorization numbers 11,141 and 13,474.

ment at zero time with reference to radioactivity decay. This ratio, which is proportional to the K^{42} concentration, was computed by the expression

$$K_0/I_0 = \frac{D\left[\frac{S_1}{t_1} - \frac{S_3}{t_3} - d\left(\frac{S_2}{t_2} - \frac{S_4}{t_4}\right)\right]}{d\left(\frac{S_2}{t_2} - \frac{S_4}{t_4}\right)} = \frac{D \cdot K_u}{d \cdot I_u}, \qquad (1)$$

where S_1 is the number of counts recorded from a paper segment during the first counting time t_1. It is the sum of the counts contributed by K^{42}, I^{131}, and background. S_2 is the number of counts recorded in time t_2 from the same paper segment 5 days later when effectively all the K^{42} has decayed. This is the sum of the counts of the partially decayed I^{131} and background. S_3 and S_4, counts recorded over time t_3 and t_4, are backgrounds taken as proper corrections for S_1 and S_2. The factor d corrects for the decay of I^{131} over the 5-day interval, thus adjusting S_2 back to the time of S_1; D corrects for the decay of K^{42}, thus adjusting the initial K^{42} count back to zero time. K_u and I_u are the counting rates of K^{42} and I^{131}, respectively, corrected for everything except their own decay.

The standard error in the determination of K_0/I_0 is given by the expression:

$$\sigma_{K_0/I_0} = K_0/I_0 \sqrt{\frac{S_1/t_1^2 + S_3/t_3^2 + d^2(S_2/t_2^2 + S_4/t_4^2)}{K_u^2} + \frac{S_2/t_2^2 + S_4/t_4^2}{I_u^2}} \qquad (2)$$

The plus and minus value of the standard error is marked by a vertical bar above and below each experimental point in Figs. 1 and 2. For a given K^{42} counting rate and given rates of the two backgrounds, the optimum rate for the I^{131} count for minimum counting error is given by solving Eq. (3) for I_u and correcting for decay.

$$I_u^3 d(1/S_1 + 1/S_2) + I_u^2\left(\frac{K_u + S_3/t_3}{S_1} + \frac{dS_4/t_4}{S_2}\right) - \frac{K_u^2 S_4/t_4}{dS_2} = 0. \qquad (3)$$

The derivation of Eq. (3) assumed either that the background S_4 is counted long enough that $S_4 = S_2$ or that background is sufficiently smaller than the I^{131} counting rate that the square of the background is negligibly small with respect to I_u^2.

Minor fluctuations in the dotted line in Fig. 1 are not to be taken as significant. Their existence, if real, suggests a difference in circulation times among various regions of the vascular bed. The time between the onset of systole and the successive rises in K^{42} concentration may be attributed to the travel time of the fluid between the capillaries and the collection site. Changes in the amounts of K^{42} counted are the source of the fluctuations, since the I^{131} count for volume remains fairly constant. There is, however, an over-all increase in flow at the end of systole which is offset by an even larger increase in the amount of the released K^{42} in the vascular fluid. Refinements in technique that will permit corrections for travel time and for individual variations in the geometry of the vascular bed are now being developed. These refinements and the physiological interpretations that they permit will be reported at a later date.

We further visualize an effluochromatogram. The outflow might be caught along one edge of a filter paper rectangle. The effluent chemicals from the muscle, now dispersed horizontally in time along the length of the paper, might then be induced to migrate vertically on the paper by the method of chromatography. Chemicals all emitted from the tissue at a given time could be related as to quantity. If two chemicals were adsorbed together on the chromatogram, it would be suspected that chemical association or complexing was present as they emerged from the tissue. A more onerous task would be to attempt two-dimensional chromatography—horizontal displacement by chromatography of chemicals that had previously migrated vertically above each time segment.

Ginsburg and Wilde (*1*) have used the method to construct the mixing curve for K^{42} in the circulation after injection into the femoral vein of rats. This is the "indicator dilution curve" used to measure cardiac output by the single injection method (*2*). Arterial blood was led onto the filter paper strip from polyethylene tubing cannulated into the carotid artery. The volume of blood collected onto each paper segment was estimated from radioactive iodinated plasma albumin injected and equilibrated in the rat circulation ahead of time. By this method points were recorded every 0.1095 sec (Fig. 2), whereas in earlier work (*2*) 2-sec intervals were required. The irregularity to the right of the initial peak might be due to the continuing injection, which began at −4.7 and ended at +0.3 sec. If the injection can be accomplished in a shorter time, fluctuations indicative of possible arterial-venous shunts in the lungs or of recirculation through the coronary system of the heart may be found.

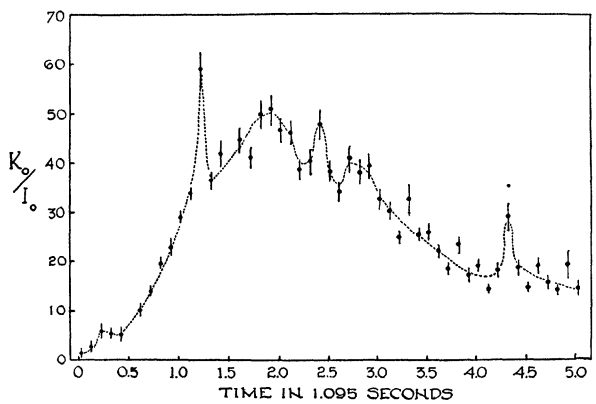

FIG. 2. Effluoradiogram for carotid arterial blood after injection of K^{42} into the femoral vein of a rat.

References

1. GINSBURG, J., and WILDE, W. S. Unpublished observations.
2. SHEPPARD, C. W., *et al.* In preparation.

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