Diffusion Processes Invalidating the Intra-arterial Krypton-85 Beta-Particle Clearance Method for Measurement of Skin Blood Flow in Man

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ABSTRACT
Krypton-85 dissolved in 0.9% NaCl was injected into the femoral artery of man; β particles were measured over the skin of the crus. Cutaneous mean blood flow was calculated using the integral equation of Zierler based on the mean transit time analysis of clearance curves recorded from the cutaneous tissue; these calculations gave estimates which were only about one-fifth those obtained by other methods. The low estimates were caused by an intercompartamental exchange of 85krypton between the cutaneous and subcutaneous tissues, an exchange implying that both tissues influence the shape of the clearance curve recorded from the cutaneous tissue alone. This intercompartamental exchange was effected not only by diffusion directly from the cutaneous to the subcutaneous tissue, but presumably also by transport from the blood streaming between the two tissues. The clearance rate of the tail of the curve was shown to correspond to that of the subcutaneous tissue. The errors distorting the clearance curve of β particles may invalidate the use of intra-arterial injection and measurement of clearance curves of β particles for measurement of blood flow in other tissues.

ADDITIONAL KEY WORDS
inert gas diffusion
tracer clearance models
intercompartamental exchange
clearance of β particles
compartmental analysis of clearance curves
diffusion processes between cutis and subcutis
mean transit time analysis of clearance curves

The radioactive inert gas 85krypton has been used as a tracer in the measurement of skin blood flow in dogs by Bell and Harper (1) and in rabbits by Casey and Thorburn (2), and Thorburn, Casey and Molyneux (3). These studies represent applications of a method devised by Lassen and Ingvar (4) for the measurement of local blood flow through the cerebral cortex. This method is based on intra-arterial injection of 85krypton dissolved in a 0.9% saline solution with external recording of the β-radiation which penetrates only the superficial layer of ca. 1 mm thickness.

The present study uses these principles in an attempt to develop a quantitative method for measurement of skin blood flow in man.

Material and General Experimental Conditions
The experiments were performed on normal subjects ranging in age from 24 to 76 years who were placed in the recumbent position at an ambient temperature of 19 to 22°C and a relative humidity of about 60%, i.e. nonsweating conditions. The subjects were dressed with the exception of the leg from which blood flow was to be measured. The measuring area was the lateral surface of the crus and in a few cases the dorsum of the foot.

INTRA-ARTERIAL 85KRYPTON CLEARANCE
The inert gas 85krypton (Radiochemical Centre, Amersham, England) dissolved in 0.9% NaCl, ca. 2 mc/ml was used. 85Krypton solution (2 to 6 ml) was injected into the femoral artery. The injections lasted 3 to 5 sec. A total of 16 determinations on 16 subjects was performed.
The $^{85}$krypton $\beta$ particles were measured by a Geiger-Müller tube, 32 mm in diameter, placed 5 mm from the skin. The Geiger-Müller tube was coupled to a scaler connected with a digital printer so that the cumulative counts in 1-min intervals could be recorded. The clearance curve was plotted semilogarithmically against time.

**Theoretical Considerations**

$^{85}$Krypton emits mainly $\beta$ particles (maximal energy 0.67 Mev) and because of the self-absorption in the tissue counting rates are reduced. A tissue layer of 0.25 mm will reduce the counting rate by 50% (5). This means that if the activity is distributed uniformly in the tissue, about 90% of the $\beta$ particles recorded will originate from the superficial 0.85 mm. The thickness of the skin on the crus and the dorsum of the foot is more than 1 mm. This means that for all practical purposes the radiation measured is emitted from $^{85}$krypton located in the skin alone, and that the superficial layers of the skin are recorded with much higher efficiency than the deeper layers.

Blood flow has recently been calculated for the cerebral cortex (4, 6), and for the superficial layer of the myocardium (7) from curves of the washout of the residue of tracer $^{85}$krypton ($^{85}$krypton $\beta$-particle clearance) following a rapid intra-arterial injection. In both situations, use is made of the mean transit time analysis proposed by Zierler (8) showing that:

$$ t = \frac{\text{Area}}{\text{Height}} = \frac{\text{Total number of impulses under the curve}}{\text{Maximum counting rate}} $$

(1)

where $t$ is the mean transit time of particles in the system. Another expression for $t$ is the volume to flow ratio, $V/F$

$$ t = \frac{V}{F} = \frac{\lambda}{f} $$

(2)

where $f$ is flow in milliliters per gram per minute, and $\lambda$ is the tissue-to-blood partition coefficient as defined by Kety (9), i.e. it is the equilibrium ratio $C_{\text{tissue}}$ to $C_{\text{blood}}$ of the indicator.

Since $C_{\text{tissue}}$ is customarily measured in counts per minute per gram and $C_{\text{blood}}$ is measured in counts per minute per milliliter, then $\lambda$ has the unit of milliliters per gram and expresses the volume of distribution of $^{85}$krypton in milliliters of blood per gram of tissue. $\lambda$ depends on the percentage of water, saline, protein and fat of the two phases and can be estimated to be close to 1.0 in all parenchymatous tissues, muscle and skin, while it is about 5.0 in adipose tissue (10, 11).

Eliminating $t$ by inserting equation 1 in 2 yields

$$ f = \lambda \cdot \frac{\text{Height}}{\text{Area}} \text{ ml/g \cdot min} $$

(3)

The "height" of the clearance curve, the maximum counting rate in counts per minute, must be a relative measure of the total amount of $^{85}$krypton entering the skin via the blood. It is therefore of essential importance for the method that the entire bolus reaches the skin by the arterial blood before any loss has taken place (12). The bolus of $^{85}$krypton is here defined as the amount of the indicator gas which originally, i.e. in the femoral artery, was contained in the fraction of the blood perfusing the skin area that was measured.

The "area" under the curve is the cumulative amount of $^{85}$krypton activity recorded during the entire washout process of the bolus by the blood perfusing the skin, i.e. no other routes of clearance should exist such as loss by diffusion through the skin surface or loss down into and clearance from the subcutaneous tissues. The "area" was calculated as a sum of the integrated area under the measured curve, and the area of the tail was extrapolated to infinity as a monoexponential function with the half-time of the measured tail.

Casey and Thorburn (2) and Thorburn, Casey and Molyneux (3) used a formula for calculating mean skin blood flow in the rabbit following the injection of a slug of $^{85}$krypton which takes the form of the weighted average of the estimated blood flow of two compartments in-parallel within the skin. However, as has been shown elsewhere (12),
this model represents merely a special case of the mean transit time of "noncompartmental" analysis of Zierler and the equation of Casey and Thorburn can be reduced to equation 1 by simple algebraic manipulation.

Bell and Harper (1) employed a 4-min injection time in their studies of skin blood flow in dogs using intra-arterial $^{85}$krypton injection. Assuming that an uniform labeling of the skin has thus been achieved, it can be shown that the relative initial slope and not the relative area allows one to estimate mean tissue blood flow (5). Thus, the approach of Bell and Harper is, in principle, the same as that of Casey and Thorburn when one takes into account the different mode of tracer injection.

In addition to estimation of mean skin blood flow, Casey and Thorburn (2) also tried to evaluate the flow and the relative mass of the two compartments. Thorburn, Casey and Molyneux (3) found the fast compartment dominating in areas with active hair growth, and they concluded that the fast compartment represented the blood supply to follicular tissue and the slow compartment was the supply to the rest of the skin.

In the study of skin blood flow in man presented later, this graphical resolution is not employed since no anatomical structures could be found in the cutaneous tissue examined that would correspond to the two widely different flow rates (the hair growth in the skin area under study was minimal).

**Results**

Figure 1 shows a typical curve from vasodilated warm skin (skin temperature 32°C) after an intra-arterial injection. It is remarkable that the maximum count rate is reached as late as 4 min after the injection, which takes only a few seconds. This must be compared to the passage time for blood plasma from the site of injection in the femoral artery to the measuring area on the middle of the crus which can be estimated to be of the order of only 4 to 5 sec (13). Consequently, the top of the curve should have been expected after only 7 to 10 sec if the $^{85}$krypton has stayed in the blood.

Twelve experiments were performed on vasodilated skin (skin temperatures ranging from 30.5 to 33.5°C). In all the experiments, the delays to the top of the curve were found to exceed 3.5 min, average 5.5 min, SD 1.4.

In 4 experiments with "cold" vasoconstricted skin (skin temperatures from 27 to 29.5°C), the maximum counting rate of the curve was found even later as seen in Figure 2. The delay in these 4 experiments was 16, 21, 45, 60, 90, 120 min.

![Figure 1](http://circres.ahajournals.org/)}
and 75 min, average 39 min. The curves in Figure 2 do not show the initially fast increase and decrease in counting rate seen in Figure 1.

It should be emphasized that the room temperature was the same in both series of studies and that the subjects had no subjective feeling of heat or cold. It was only by recording the skin temperature that the two different states could be defined.

The blood flow calculated from the height/area equation in 6 experiments on vasodilated skin averaged 1.1 ml/100 g·min, SD 0.4, and in 3 experiments on vasoconstricted skin, 0.34 ml/100 g·min, SD 0.14. The calculation was performed only for the 9 experiments in which steady state was maintained and in which the curves were followed sufficiently long to give a well-defined monoexponential tail.

In all 16 experiments, both on vasodilated and on vasoconstricted skin, the curves eventually (after about 1 hr) reached approximately the same slow rate of decrease which continued monoexponentially in the following hours (maximum time of observation was 5 hr, see Fig. 2). The half-time of the tails in these 9 experiments with well-defined monoexponential tails averaged 168 min, SD 41. No difference was noted between the half-time of the tails in vasodilated and vasoconstricted skin.

**COMMENTS**

As already mentioned, a basic assumption for this mode of calculation is that the entire bolus of 85 krypton reaches the skin before any significant loss has occurred. The experiments strongly suggest that this assumption cannot be fulfilled, as the marked delay of the maximum counting rate (3 ½ to 75 min) is much longer than the intravascular transit times can explain. Furthermore, doubt must be raised as to what extent the area under the clearance curve really represents—only the washout by the blood perfusing the skin. The experiments that follow were designed to...
elucidate these problems. They show that the values already given must be a gross underestimation of skin blood flow. The possible significance of the various components of the clearance curves will also be analyzed on the basis of these supplementary experiments.

**Supplementary Studies**

Since the input of $^{85}$krypton continues for at least 3% to 75 min, it is of interest to evaluate the shortest clearance time of $^{85}$krypton molecules that have already reached the skin: If this time is shorter than the delay time, then the maximum height of the curve cannot represent the entire bolus.

**Local Injection of $^{85}$Krypton in Saline and Recording of $\gamma$ Rays**

$^{85}$Krypton (0.05 to 0.01 ml) in isotonic saline was injected intracutaneously on the crus. The injections were performed using needles with 0.25-mm o. d. and the duration of the injections was about 5 sec. The low incidence of $\gamma$ rays from the $^{85}$krypton depot was measured by a scintillation detector (NaI [Tl] crystal) covered with a sheet of 2-mm plexiglass to avoid interference from the high incidence of $\beta$ particles (99.4% of emissions).

The detector was coupled to a ratemeter with a time constant of 1 sec in connection with a linear potentiometer writer. The clearance curves were plotted semilogarithmically against a time scale of minutes.

The clearance curves showed a fast initial decrease of the counting rate which gradually passed into a slow decrease. The decrease in all experiments was maximum immediately after the injection (10 experiments, see Fig. 3). Therefore, the shortest clearance time for $^{85}$krypton deposited in the skin is in the order of a few seconds.

**Comments**

The variability and the very fast initial decrease rate of the clearance curves in these experiments suggest that the trauma of injection disturbs the local circulation. But, it may safely be assumed that the spontaneous (non-traumatized) clearance curve is, in principle, similar, i.e. that it has the same general shape showing a clearance of $^{85}$krypton starting immediately after the injection. The results presented above thus show that the shortest washout time for $^{85}$krypton deposited in the skin by an intra-arterial injection is in the order of a few seconds and that a considerable loss of the tracer has occurred before the maximum counting rate of the intra-arterial $\beta$-particle curves is reached. The maximum height of the curve, therefore, underestimated the bolus in all cases. But what factors cause the delay?

**Local Injection of $^{85}$Krypton in Saline and Recording of $\beta$ Particles**

$^{85}$Krypton (0.005 to 0.100 ml) in isotonic saline was injected intracutaneously on the crus. The injections were performed as already described and the $\beta$ particles emitted were measured by a Geiger-Müller tube. In some experiments the circulation was arrested by a tourniquet placed proximal to the knee joint. The pressure in the cuff was held about 50 mm Hg above the systolic pressure.

The $\beta$-particle clearance curve obtained from such an intracutaneous depot showed an initial increase of the counting rate. The maximum of the curve occurred after an average
Local injection of \textsuperscript{85}krypton and recording of \(\beta\) particles.

The delay of the maximum counting rate after an intracutaneous injection must be due to diffusion of \textsuperscript{85}krypton towards the Geiger-Müller tube. A diffusion barrier to inert gas has been demonstrated in the middle one-third of the epithelium (14). It is therefore likely that this diffusion mainly takes place towards the demonstrated barrier. The increase of the counting rate in spite of arrested circulation also indicates that diffusion processes cause the phenomenon.

Even more surprising than the increase of the counting rate was the finding of a fairly rapid subsequent decrease in the experiments with arrested circulation. This phenomenon cannot be due to diffusion through the barrier (already mentioned) or to loss by sweat. Nor can it be the effect of incomplete circulatory arrest. All routes of loss from the area were excluded by the finding of practically complete cessation of all clearance when using \(\gamma\)-ray counting instead of \(\beta\)-particle counting. On this basis it is concluded that the decrease in \(\beta\)-particle counting rate during application of tourniquet is due to diffusion from the cutaneous layer to the subcutaneous tissue. In radioautographic studies with \textsuperscript{133}xenon, to be reported elsewhere, this process is readily demonstrable.

Can the delayed maximum observed in vasodilated skin after intra-arterial injection be explained entirely by the delays seen after local injection? If this was the case, then the tracer must have reached the skin as a bolus of very short duration corresponding to
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INTRA-ARTERIAL INJECTION OF $^{85}$KRYPTON AND RECORDING OF $\beta$ PARTICLES (UPPER CURVE). INTRA-ARTERIAL INJECTION OF $^{85}$KRYPTON AND RECORDING OF $\beta$ PARTICLES, EFFECT OF Tourniquet BEFORE THE MAXIMUM OF THE CURVE (LOWER CURVE).

Two experiments performed on the same leg. Intra-arterial injection of $^{85}$krypton and recording of $\beta$ particles (upper curve). Intra-arterial injection of $^{85}$krypton and recording of $\beta$ particles, effect of tourniquet before the maximum of the curve (lower curve).

The few seconds that the local injection lasts. The longer duration of the delay in the intra-arterial curves suggests that the arrival of the bolus to the cutaneous tissue must be extended to about 2 min rather than a few seconds.

Further studies in vasodilated skin support the idea that the intra-arterial bolus of $^{85}$krypton is extended over some time.

EFFECT OF INTRA-ARTERIAL INJECTION OF $^{85}$KRYPTON WITH RECORDING OF $\beta$ PARTICLES AND APPLICATION OF Tourniquet BEFORE THE MAXIMUM OF THE CURVE

A tourniquet was placed proximal to the knee joint 20 sec after an intra-arterial injection in the femoral artery. The $\beta$ particles were measured over the lateral surface of the crus. The tourniquet was maintained for 15 min. The experiment which was performed on vasodilated skin (temperature about 32°C) resulted in a curve shown in Figure 6, lower curve. The increase of the counting rate continues in spite of arrested circulation (diffusion towards the epidermal barrier). A maximum is reached after 6 to 7 min followed by a very slow rate of decrease. After release of the cuff, a second maximum occurs and hereafter follows a decrease almost similar to that of the undisturbed intra-arterial curve from the same leg seen in Figure 6, upper curve.

Comments

The time interval of 20 sec from the start of the injection till the inflation of the tourniquet would have permitted the "real" arterial bolus, i.e. the albumin and red cells initially mixed with the $^{85}$krypton, to arrive practically in toto to the cutaneous tissue.

The second increase of the counting rate after release of the cuff indicates arrival of a substantial fraction of the bolus. This late arrival of a fraction of the bolus points to a volume of distribution of $^{85}$krypton "before" the skin comprising not only the blood but also the arterial walls and perhaps the immediately surrounding tissue. This model implies a possibility of irreversible loss of some part of the bolus and also that $^{85}$krypton may be transported from one tissue to another by entering the arterial (or venous) blood streams.

Clear evidence of $^{85}$krypton leaving the blood "before" it reaches the cutaneous layer is afforded by the intra-arterial $\beta$-particle clearance curves in the moderately vasoconstricted skin. In this situation no bolus of indicator can be discerned as the curve rises slowly over ½ to 1 hr. It is therefore considered likely that the same type of loss through the arterial walls also occurs to some degree in the vasodilated skin, so that a fraction of the bolus is lost as already discussed.

Besides diffusion through the walls of the vessels, bulk diffusion between the cutaneous and the subcutaneous tissue is of importance. This will be demonstrated by the following experiments.

EFFECT OF INTRA-ARTERIAL INJECTION OF $^{85}$KRYPTON, WITH RECORDING OF $\beta$ PARTICLES AND APPLICATION OF Tourniquet AFTER THE MAXIMUM OF THE CURVE

In some experiments with intra-arterial $^{85}$krypton $\beta$-particle technique on vasodilated

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skin, the circulation was arrested by a tourniquet at various times after the maximum of the curve. When the tourniquet was applied during about the first 45 min, the clearance did not stop completely (see Figure 7, left side). The decrease of the counting rate in spite of arrested circulation was most pronounced shortly after the maximum of the curve, declining with time, so that the curve was horizontal during application of the tourniquet after about 45 min. If the tourniquet was applied after more than 1 hr, the counting rate was found to increase (see Figure 7, right side).

**Comments**

The decrease of the counting rate in the first hour of clearance is, as previously mentioned, due to diffusion in the depth. In these experiments the effect of the diffusion in the depth was smaller than in the intracutaneous experiments probably because the subcutaneous tissue also receives $^{85}$krypton by the arterial blood.

The increase of the counting rate observed when arresting the circulation after 1 hr is evidence for the diffusion of $^{85}$krypton up from deeper layers of the skin. This process must also take place when the blood is flowing, and hence it constitutes a continuous input into that superficial layer of the skin which is counted, i.e. tracer arrives long after the maximum of the curve. This continued influx of tracer is presumably mainly due to internal recirculation, i.e. $^{85}$krypton having been deposited in the subcutaneous layer by diffusion (directly and through the walls of the veins) during the early part of the experiment. This means that the same indicator particles are recorded more than once. It decisively invalidates the use of the height/area equation since the area then will be too great. As already commented on, the height is too small to give an estimate of the total input of tracer to the skin. These two errors thus both result in an underestimation of the blood flow if one erroneously attempts to use that equation.

Recirculation of $^{85}$krypton via the lungs also could cause a falsely great area, but recording over the symmetrical region on the other leg has not shown a measurable recirculation after intra-arterial injection with the technique used.

The increase of the counting rate when the circulation is arrested more than 1 hr after the injection is possible only if the deposition of $^{85}$krypton is very close to the skin, so that the tracer can reach it by diffusion. That
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$^{85}$krypton really can reach the skin from the subcutaneous tissue immediately under the skin was shown by the following experiment.

**SUBCUTANEOUS INJECTIONS OF $^{85}$KRYPTON, RECORDING OF $\beta$ PARTICLES AND $\gamma$ RAYS**

A subcutaneous depot of $^{85}$krypton in saline, 0.05 to 10 ml, 2.0 to 0.1 mc/ml, was injected just under the skin on the crus. A long injection needle was introduced through subcutaneous tissue for a distance of 15 cm to the point of injection. The $\beta$ particles were recorded in two experiments where a tourniquet was applied at various intervals after the injection (see Fig. 8). This curve resembles that from vasoconstricted skin after intra-arterial injection seen in Figure 2. The increase of the counting rate during the first hour which occurs in spite of arrested circulation shows that $^{85}$krypton can reach the measuring area in the skin from the subcutaneous tissues by diffusion. In addition the curve in Figure 8 shows that immediately after the injection no $\beta$ particles are recorded, confirming that only $\beta$ particles originating from the skin can be detected.

In two other experiments, the $\beta$ particles and $\gamma$ rays were measured alternately by a Geiger-Müller tube (see Fig. 9). The recording of $\gamma$ rays was performed by interposing a sheet of 2-mm plexiglass between the tube and the skin. The $\beta$-particle and $\gamma$-ray curves reached the same slow rate of decrease about 1 hr after the injection.

**Comments**

These experiments indicate that the tail of the $\beta$-particle clearance curves recorded from the cutaneous tissues after subcutaneous injection show the same slow clearance rate as the subcutaneous tissue represented by the tail of the $\gamma$-ray clearance curve. Presumably the clearance rate in the tail of the $\beta$-particle curves (cutis) is an expression of the slow

![Figure 8](http://circres.ahajournals.org/)

**Figure 8** Subcutaneous injection of $^{85}$krypton and alternately recording of $\beta$ particles and $\gamma$ rays.

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decrease in the concentration of $^{85}$krypton in the subcutaneous tissues from which the $^{85}$krypton is transported to the cutis either by diffusion or by the blood. In other words, an in-series arrangement of the two tissue compartments is suggested by this finding.

If one calculates the blood flow from the half-time of the tail of the curves using the partition coefficient $\lambda = 5.0$ for fat tissue, a value of about 2.0 ml/100 g • min is obtained. This corresponds to the estimation of subcutaneous blood flow determined by $^{133}$xenon and $^{85}$krypton in the deep subcutaneous fat of the abdominal wall (11).

The in-series arrangement presented here predicts that the concentration of $^{85}$krypton in the skin is the net result of a balance between influx and efflux. This balance was clearly shown by the following experiment.

**EFFECT OF INTRA-ARTERIAL INJECTION OF $^{85}$KRYPTON, LOCAL HISTAMINE AND APPLICATION OF TOURNIQUET ON THE TAIL OF THE CURVE**

$^{85}$Krypton was injected intra-arterially and the $\beta$ radiation was recorded. Histamine was rubbed on the skin over the crus when the clearance curve had reached the tail, i.e. about 1 hr after injection. The skin was then scratched with a needle. This procedure produces a cutaneous hyperemia. After this the circulation was arrested by a tourniquet for a period and the procedures were repeated (Fig. 10). The curve shows a sudden fast decrease provoked by the histamine hyperemia and a rise effected by the application of the tourniquet.

**Comments**

Alterations in the cutaneous blood flow will thus influence the clearance rate and disturb the balanced clearance conditions during the tail of the curves.

**General Discussion**

The considerations mentioned point out several errors influencing calculation of the average skin blood flow in man from the clearance curve of $^{85}$Kr after intra-arterial injection. These errors arise from diffusion processes by which $^{85}$krypton is exchanged between subcutaneous and cutaneous tissues. Intercompartmental exchange has previously been calculated by Perl et al. These authors assumed that the exchange between adjacent tissue compartments was only by diffusion across the boundary between tissues (15-19) and not by transport via blood streaming between tissues. Observations in the present study, however, indicate that such a blood transport mechanism is also of importance. The errors caused by the intercompartmental exchange affect both the mean transit time analysis (equation 1) as well as the two compartmental analysis of Thorburn et al. (2, 3),
which, as already mentioned, can be considered merely as a special case of the mean transit time analysis when used for estimating mean blood flow. The important question then arises: how great are these errors; can they be neglected, or do they grossly distort the results?

In the following discussion this question is answered through a comparison of the results obtained from the $^{85}$krypton $\beta$-particle clearance curves calculated from the mean transit time analysis with the results of other methods for determining skin blood flow.

The estimated skin blood flow was as mentioned 1.1 ml/100 g min in vasodilated skin at an environmental temperature of 19 to 22°C determined by the $^{85}$krypton $\beta$-particle counting technique.

Using heat loss measurements (calorimetry), Hardy and Soderstrom (20) estimated the total cutaneous blood flow at an environmental temperature of 23°C in a nude subject at rest. The cutaneous blood flow was 80 ml/m$^2$ body surface min. Under similar conditions the helium uptake from the skin on the trunk and the extremities was studied by Behnke and Willmon (21); an average value obtained from their data is 75 ml/m$^2$ body surface min (average of three determinations). Assuming that the mean thickness of the skin is 1.5 mm, the blood flow will be about 5.1 and 4.5 ml/100 g min respectively.

Skin blood flow has been estimated as the difference in forearm blood flow before and after iontophoresis of adrenaline into the skin by Cooper et al. (22), and by Kontos et al. (23); they used a water-filled venous occlusion plethysmograph with water temperatures of 30 to 35 and 33°C respectively. The room temperatures ranged from 19 to 27°C and from 23 to 24°C in the two studies. A greater scatter of the results was found by Cooper et al., but if we exclude the five extremely low and the five extremely high values and assess cutaneous tissue as 8.6% of the forearm tissue volume, the average of the remaining 21 experiments was 11 ml/100 g min ± 8.7 (recalculated in accordance with Cooper et al.). A very important observation was made by Kontos et al. by inserting a thin catheter in a superficial vein which drained only cutaneous and subcutaneous tissues: following iontophoresis no venous blood could be collected from the catheter (4 experiments). This was not due to obstruction of the catheter, for when the effects of adrenaline wore off, a free flow of blood could be obtained. It can be concluded that the flow measured by the iontophoresis technique is probably a sum of the cutaneous and the subcutaneous blood flow, i.e. "skin" in this connection means cutis plus subcutis. Cooper et al. found that the subcutaneous tissue constitutes a similar percentage of the forearm volume as compared with the cutaneous tissue. The mean blood flow in cutis plus subcutis in these two studies will then be 4.4 and 5.5 ml/100 g min respectively. As the blood flow in the subcutaneous tissue is estimated to about 2 to 3 ml/100 g min (11), then the most likely values for the cutaneous tissue here will be about 6 to 9 ml/100 g min.

Using $^{133}$xenon applied by the nontraumatic epicutaneous technique (24) under the same conditions and in the same region (on the crus) as in the present paper, it can be estimated that values for the cutaneous blood flow were 5.7 ml/100 g min, ± 1.15 in 10 experiments (24).

The estimates presented above for the cutaneous blood flow are thus in fair agreement with each other and they contrast sharply with the values obtained using the intra-arterial $^{85}$krypton $\beta$-particle technique. It is concluded that the $^{85}$krypton $\beta$-particle technique under the conditions of study employed here yields an estimate of only about one-fifth of that indicated by other methods.

It could be postulated that this low estimate could be partly explained by the existence of arteriovenous shunting of the blood in the skin, but no arteriovenous shunts have been found in the skin of the extremities except for the volar surfaces of the hand and foot and at the nails (25).

Three studies based on concepts of the same nature as in the present study (intra-arterial injection of $^{85}$krypton and recording...
of β particles over the skin have recently been reported in the literature. Bell and Harper (1) found monoexponential clearance curves in anesthetized dogs after injections (duration, 4 min) (only recorded for the first 15 min). These curves had half-times of 21 min corresponding to a cutaneous blood flow of 3.3 ml/100 g • min.

Casey and Thorburn (2) and Thorburn, Casey and Molyneux (3) using a slug injection found in anesthetized rabbits a bi-exponential clearance curve (recorded for 40 to 50 min). Both compartments were considered to originate from the skin. They estimated the blood flow of these two compartments to be about 30 ml and 3 to 4 ml/100 g • min. Their curves show no delay of the maximum counting rate except in a few cases with reduced body temperature where the maximum was reached about 20 min after the injection. In experiments where the animals were killed shortly after the intra-arterial injection, the decrease in the counting rate was found to be extremely small and insufficient to affect the results.

In these studies on animals, the calculation of the cutaneous blood flow is presumably not subject to the gross errors demonstrated in the present study in man. The low fat content in the subcutaneous tissue in lean dogs and rabbits would cause a smaller effect of the intercompartmental exchange. However, such exchange also exists in those animals that had the maximum counting rate delayed for 20 min. How much this exchange influences the results from the vasodilated skin in dogs and rabbits is uncertain.

The demonstrated difficulties involved in the use of the 85 krypton β-particle technique on the skin in man invalidate its application for this purpose, but the technique yields a possibility of recording the subcutaneous clearance rate after an atraumatic injection by using the monoexponential tail of the curve. One could imagine that the clearance in the subcutaneous tissue would be faster in the superficial layer because of the opportunity for the 85 krypton molecules to escape by diffusion to the adjacent cutaneous tissue which offers much faster clearance conditions, but this is probably without importance.

Diffusion processes may be of importance when the β-particle clearance method using 85 krypton is used to measure blood flow of other tissue surfaces. The skin may be considered an extreme example of a "hilus organ," i.e. an organ in which the blood flow is centrifugal from a hilus. In such an organ, freely diffusible tracers, e.g. gases, will have the opportunity to leave the blood before the latter reaches the surface, i.e. to exchange in a compartment placed "before" the surface. The liver may serve as an example: the injection of a slug of 85 krypton into the portal vein is followed by a markedly delayed maximum counting rate (15 to 35 sec) as measured by a Geiger-Mueller tube placed over the surface (26). In this case the same difficulties as discussed for the measurement of cutaneous blood flow apply, resulting in an underestimation of tissue blood flow by using the mean transit time analysis (26). In the kidney the same type of errors must in principle exist and it is therefore interesting to note the fairly low values for renal cortical blood flow obtained using 85 krypton and β-particle clearance (27) even when the tail of the clearance curve is not included in the calculation.

In organs which do not have such a hilus, the diffusion processes would appear to be of lesser importance. Indeed, if the tissue is completely homogeneous, then the β-particle clearance method should be strictly valid. A comparison of myocardial blood flow measured by β-particle and γ-ray techniques was performed by Doutheil and Rohde (7) on dogs. They found a fairly good correlation of the results from these techniques, but a rather large scatter. In only 2 of 8 cases was a significant correlation found between the results with the β-particle technique and directly measured flow, but a significant correlation was found in 6 out of 8 cases with the other technique. Apparently the myocardium is not an ideal homogeneous tissue for application of the β-particle technique.

In the brain the blood is distributed from the surface to the cerebral cortex and the subj-
cortical white matter. Ingvar and Lassen (5) and Haggendal, Nilsson, and Norback (28) employed the $\beta$-particle clearance method for measurement of mean blood flow in the cerebral cortex in cats and dogs. When fitting the recorded curves, two or three exponentials were necessary. The slowest compartment had a clearance rate of the same order as that obtained by local injection in the white matter when recording $\gamma$-rays. By recording $\beta$ particles over the cortex after local injection in the white matter 4 mm in depth, no activity was observed initially, but then an accumulation was recorded. A maximum was obtained after $\frac{1}{2}$ to 1 min and the clearance rate thereafter corresponded to that of white matter, demonstrating diffusion of the tracer from the white to the grey matter. By arresting the circulation immediately after an injection of $^{85}$krypton into the internal carotid artery of a cat it was demonstrated by Brock (personal communication, 1967) that diffusion from grey to white matter occurred. The brain surface was covered by a gas tight Mylar membrane to avoid loss of $^{85}$krypton from the surface. The decrease of the counting rate in spite of the arrested circulation was 10% of the undisturbed initial rate of decrease. If the circulation was arrested 10 min after the injection (on the tail of the curve) an increase of the counting rate was observed. Thus there is some degree of diffusion between the grey and white matter in the brain.

These diffusion processes also influence the two compartment in-parallel model used for analysis of the clearance curves of $\gamma$ rays in the brain as recorded after an injection of a slug of $^{85}$krypton or $^{133}$xenon in the internal carotid artery (12). The experimental data obtained in normal man yields a somewhat exaggerated estimate for the relative weight of the white matter (12, 29) when applying the strictly in-parallel model previously used. The bi-directional exchange (by diffusion and also by transport with the blood) occurring between adjacent parts of the grey and white matter means that, among other things, the relative weight of the white matter will increase. The quantitative significance of these diffusion processes is thus supported by the known data. With regard to the renal medulla such exchange processes (here named counter current diffusion) are known to be of major importance (30, 31), rendering virtually meaningless the three (or four) homogeneous compartments in-parallel model (32).

It should be stressed that exchange of diffusible tracers between tissue compartments does not affect the calculation of mean blood flow from external $\gamma$-ray clearance curves from a single tissue: The mean blood flow obtained by the mean transit time analysis does not necessitate diffusion equilibrium, but only isoefficiency counting, i.e. the different tissue elements must be represented equally in all depths of the tissue (8). An experimental verification of this method as applied to the isolated gastrocnemius muscle in cats has recently been made by Tønnesen and Sejersen (33).

With regard to the human skin, a physiological consequence of the observed diffusion exchange of $^{85}$krypton must be that heat, which diffuses much more rapidly than gas, will be exchanged very effectively. Efficient heat exchange, as previously found by Bazett et al. (34) in larger arteries and veins of the forearm, and by Aukland (35) in the medulla of the kidney, presumably also takes place in the subcutaneous tissue and in the cutaneous plexus, enabling the organism to counteract the heat loss from the skin surface.

Acknowledgment

I am indebted to N. A. Lassen, M.D., Department of Clinical Physiology, Bispebjerg Hospital, Copenhagen, for much valuable criticism and helpful discussion.

References


Circulation Research, Vol. XXI, September 1967


Diffusion Processes Invalidating the Intra-arterial Krypton-85 Beta-Particle Clearance Method for Measurement of Skin Blood Flow in Man

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doi: 10.1161/01.RES.21.3.281

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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