Blood Flow in Cutaneous Tissue in Man
Studied by Washout of Radioactive Xenon

By Per Sejrsen

ABSTRACT

Washout of $^{133}$xenon from a local depot in cutaneous tissue has been investigated in man to develop a method for blood flow measurement in that tissue. The tissue was labeled both by injecting $^{133}$xenon in saline solution intracutaneously and by a nontraumatic epicutaneous application technique. The initial selectivity of the labeling and the subsequent accumulation of $^{133}$xenon in subcutaneous tissue were examined by radioautography and by a special tissue separation technique. From the observations a two-compartment clearance model is suggested by which the cutaneous blood flow can be obtained separately from that of the subcutaneous tissue. Blood flow in the cutaneous tissue in the lateral region of the leg was 5.7 (SD 1.3) ml/100 g • min at an ambient temperature of 19° to 22°C. There were variations between different skin sites.

ADDITIONAL KEY WORDS
intercompartmental exchange by diffusion
inert gas diffusion
intercompartmental exchange by convection
trauma of injection
residue detection of $^{133}$xenon
diffusion equilibrium
compartmental analysis
effective perfusion pressure

Blood flow through the cutaneous tissue in man has not been measured quantitatively. Methods employed have been heat clearance (1-3), helium uptake through the skin (4), and plethysmography before and after adrenaline iontophoresis (5, 6). All are beset with serious sources of error, as will be commented on in the discussion. The main problem involves separation of cutaneous blood flow from that of underlying subcutaneous tissue.

The purpose of this paper is to present what appears to be a quantitative method for measurement of cutaneous blood flow in man. The method is developed on the basis of the $^{133}$xenon clearance technique for measurement of muscle blood flow (7, 8), which is a modification of Kety's local clearance method (9). In the cutaneous $^{133}$xenon clearance technique special attention is given to gas diffusion and clearance by blood flow into and away from the subcutaneous tissue. On the basis of special studies involving $^{133}$xenon clearance from cutaneous and from subcutaneous tissues individually, a solution to the analytical problem of measuring blood flow in the combined tissues is proposed in the form of a two-compartment model.

Method

Kety's local clearance method is based on injection of radioactive tracer dissolved in saline directly into the tissue. This labeling method, when applied to the human cutaneous tissue, produces hyperemia. Hence, to study the underdaged cutaneous blood flow a nontraumatic labeling procedure must be used. This can be achieved by letting $^{133}$xenon enter the cutaneous tissue through the intact epithelial surface, a method called here the epicutaneous labeling technique. As described elsewhere (10), $^{133}$xenon gas with a specific activity of 5 to 10 mc/ml of gas was introduced from a syringe into a small chamber made to adhere to the surface of a skin area from which visible hair growth had been gently cut away with a pair of scissors. In this manner a circular skin area (5 cm in diameter) was exposed to $^{133}$Xenon gas for about 3 minutes (Fig. 1). Thereafter the $^{133}$xenon gas was withdrawn into the syringe, the labeling chamber

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was removed, and surplus $^{133}$xenon was blown away. This epicutaneous labeling results in diffusion into the cutaneous tissue of a minute fraction of the $^{133}$xenon from the labeling chamber, an amount nevertheless sufficient to yield a counting rate on the order of 1 to $10^4$ counts/min with the detection system employed. "Retrograde" diffusion of $^{133}$xenon back through the skin to the environment is negligible compared to clearance with blood flow under nonsweating conditions, as has been documented previously (11) (Fig. 2).

$^{133}$Xenon dissolved in sterile saline was also injected intracutaneously in a concentration of about 0.5 mc/ml, amount 0.100 to 0.003 ml, for 0.3 to 3.0 minutes. These injections were made for comparison to the epicutaneous technique.

The detector, a sodium iodide (Tl) crystal 2.5 cm in diameter and 5 mm thick, was placed at a distance of 15 to 20 cm from the skin surface. This fairly long distance was found necessary in order to render insignificant the effect of the changing geometry of the $^{133}$xenon depot within the tissue. The scintillation detector was coupled to a scaler recording the counting rate at 0.5-minute intervals. After subtraction of background activity the clearance curves were plotted on semilogarithmic paper.

**Results**

**Comparison of Washout Curves.**—Representative clearance curves obtained by the two labeling techniques are shown in Figure 3. The atraumatic epicutaneous labeling technique gave slower initial washout rates than the intracutaneous injection technique, which suggests the effect of some trauma due to injection (10). After about 1 hour the clearance curves with both labeling techniques assumed about the same slow decrease rate. These tails of the clearance curves were studied for 1 to 2 hours, and they could in all cases readily be fitted by a straight line when plotted in a semilogarithmic diagram. In the 20 studies listed in Table 1 an average half-time of about 230 minutes was found for the washout rate of the tail of the curves. Larsen et al. (12) found almost exactly the same
Two simultaneously recorded clearance curves. The upper curve was obtained after microinjection on the one leg and the lower curve after epicutaneous labeling on the corresponding area on the other leg.

average half-time value for $^{133}$Xenon injected deep into subcutaneous adipose tissue on the abdomen in thin subjects when studying the curves 1 hour after the injections. This agreement suggests that the tail of the cutaneous clearance curve represents the clearance rate of the underlying subcutaneous adipose tissue.

Radioautographic and Microdissection Studies.—The radioautographic procedure was performed at the temperature of liquid nitrogen to minimize the diffusion of $^{133}$Xenon during the procedure. The application of the isotope was performed by injecting $^{133}$Xenon in isotonic saline (0.1 to 0.01 ml) intracutaneously or by epicutaneous labeling. The applications were done on the abdomen of anesthetized human subjects prepared for surgery. At varying times after the application, the tissue was excised and immediately frozen in liquid nitrogen. Tissue slices 50 $\mu$m thick were cut at right angles to the skin surface in a microtome of the cryostat type at $-20^\circ$C. Precooled x-ray films were placed on the frozen tissue slices and exposed for 20 to 120 hours at $-196^\circ$C. The histological slices were afterwards stained with Sudan III. Radioautographic studies were performed on tissue slices from 29 depots labeled in vivo in nine subjects. Pictures taken after 2 minutes of in-vivo clearance show with both labeling techniques a diffuse labeling of the entire thickness of the cutaneous layer but no radioactivity in the subcutaneous tissue, except for the upper part of the retinaculas of connective tissue (Fig. 4,

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Cutaneous blood flow (ml/100 g - min)</th>
<th>Subcutaneous blood flow (ml/100 g - min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epicutaneous application</td>
<td>Microinjection</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>6.7</td>
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</tr>
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<td>±SD</td>
<td>±SD</td>
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a and d). After 20 minutes of in-vivo clearance, the cutaneous content is diminished and subcutaneous localization in the adipose tissue develops (Fig. 4b). After 70 minutes of

**FIGURE 4**

Radioautograms of cutaneous (C) and subcutaneous (S) tissue. The tissue boundaries are illustrated in the schematic drawings to the right. a, b, and c were taken after injecting 0.1 ml of 133Xenon in saline intracutaneously and after 2, 20, and 70 minutes of in-vivo clearance,
in-vivo clearance, the remaining activity is located mainly in the subcutaneous adipose tissue, reaching 6 to 8 mm in the depth of this tissue (Fig. 4, c and e). The initial selective cutaneous labeling is thus lost during the clearance process. It was observed that in the

**FIGURE 4 (cont'd)**

respectively, before excision and freezing. d and e were taken after epicutaneous labeling with gaseous $^{133}$Xenon for 3 minutes. d was taken after 2 minutes and e after 70 minutes of in-vivo clearance. f was taken after intracutaneous injection of 0.1 ml of $^{133}$Xenon in saline and 70 minutes of diffusion in vitro (without blood flow). The exposure time was 20 hours in a and d, and 120 hours in b, c, e, and f.

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cutaneous part of the radioautograms the $^{133}$Xenon is at all times rather uniformly distributed within the connective tissue.

Experiments with freshly excised human skin tissue kept at 34°C showed with the same radioautographic technique that the distribution after 20 to 70 minutes was quite different from the results obtained in the in-vivo experiments. The content in the subcutaneous tissue after 70 minutes was considerably less without blood flow (Fig. 4f), and the distribution was diffuse and confined to the upper 2 mm of the subcutaneous tissue. The concentration in the cutaneous tissue was decreasing with time in the experiments with excised tissue at 34°C because of a loss of $^{133}$Xenon from the surface with sweat. This clearance rate was somewhat lower than the in-vivo clearance by blood flow, and hence the diffusion to the subcutaneous tissue without blood flow will be moderately overestimated in comparison to the diffusion with intact blood flow.

The results of these radioautographic studies in vivo and in vitro thus suggest that the transport is mainly caused by the blood flow.

Microdissection studies were performed as an attempt to quantitate the distribution of $^{133}$Xenon between cutaneous and subcutaneous tissue. The microdissection was made on a cutting plate cooled by liquid nitrogen under a dissecting microscope. Tissue slices 50 μ thick were obtained as described above. The cutaneous tissue was separated from the subcutaneous tissue by dissection with a small knife cooled in liquid nitrogen. The separated parts were then transferred to small precooled glass vials and counted in a scintillation counter of the well type. In Figure 5 data from 20 depots injected into four subjects are presented by plotting the obtained ratios between the subcutaneous counting rate and the sum of the cutaneous and subcutaneous counting rate in relation to a representative clearance curve. In this manner a time-concentration curve for $^{133}$Xenon in the subcutaneous tissue was obtained, a curve compatible with an accumulation phase followed by a slow washout phase. This microdissection method tends to slightly overestimate the values for the cutaneous tissue and correspondingly to underestimate those for the subcutaneous tissue. This is due to the difficulty of dissecting all fat from the cutaneous tissue and of making a quantitative collection of the fat cells. Furthermore it is possible that some of the subcutaneous activity has not been included in the biopsies of the size used.

Similar experiments have been performed by the epicutaneous labeling technique (six depots in two subjects). In these experiments the clearance curve was measured from the depot in vivo after about 65 minutes. Thereafter two more depots were applied with the same labeling technique 8 and 15 minutes before the excision. The result of such an experiment is shown in Figure 6.
clearance curve is thus obtained from the depot excised after 80 minutes and can strictly only be valid for this biopsy. The sources of error accounted for above seem also to influence the results in these experiments. However, in spite of the limitation of this microdissection method it supports the radioautographic findings.

Washout of $^{133}$Xenon Separately from Cutaneous or Subcutaneous Tissue.—As the $^{133}$Xenon clearance curve from an intracutaneous depot contains information of both the cutaneous and the subcutaneous clearance rates, it is tempting to consider the possibility of a resolution of the curve. It is important to know the shape of the clearance curves from either tissue alone to evaluate the basis for such a curve resolution.

The clearance curve from cutaneous tissue alone was obtained from the skin fold between the extended thumb and forefinger. This fold consists only of two layers of skin in the distal 3 to 4 mm. A lead shield 3 mm thick was made in such a way that only counting from the outer 2 mm of the skin fold was possible. Labeling was performed by the epicutaneous technique using membranes specially designed for the region. A representative clearance curve from these studies is shown in Figure 7. A monoexponential decrease was observed over more than three decades. When a scintillation detector was placed on the opposite side of the hand so that the whole of the metacarpal interspace could be seen by the probe, the clearance curve had the usual slower tail. A monoexponential clearance curve was also obtained from the dorsal region of the hand by raising a transversal skin fold through a slit in a lead shield.
The clearance process from subcutaneous tissue alone was examined in six experiments in four cats. The skin over the fatty tissue in the inguinal region was removed. A thin polyethylene film (about 10μ thick) was made to adhere to the moist surface of the adipose tissue. Care was taken to avoid all gas bubbles between the tissue and the film. Temperature was kept constant at 35° to 36°C by heat lamps. One and a half hours after the preparation was finished, about 0.1 ml of 133Xenon was placed over the polyethylene film with a small labeling chamber consisting of a plexiglass cylinder with a cannula. The edge of the cylinder was moistened with a drop of water and then placed gently on the polyethylene film. After 1 minute of labeling the cylinder was removed and surplus 133Xenon was blown away. Because polyethylene is slightly permeable, a small fraction of 133Xenon will diffuse into the underlying adipose tissue. A 20μ thick Mylar membrane which is for all practical purposes gastight was therefore placed on top of the polyethylene film with a drop of water interposed to avoid gas bubbles between the films. After this procedure counting began. The clearance curves were studied for 2 to 3 hours and were monoexponential (Fig. 8).

It is concluded that the 133Xenon clearance curves are monoexponential from cutaneous and subcutaneous tissue when these tissues are studied separately. Even though both tissues consist of a mixture of different tissue elements, the monoexponential washout of gas from each tissue points to an elimination of tension gradients by diffusion. The distances between the various tissue elements, i.e., connective tissue, sebaceous glands, hair follicles, and sweat glands in the cutaneous tissue, are at maximum 50μ to 100μ. With the known diffusion rate of 133Xenon these distances for diffusion will be negligible in relation to the duration of the washout process.

Exchange of 133Xenon between Cutaneous and Subcutaneous Tissue.—When the cutaneous and subcutaneous tissues are in their usual topographic relation, then the clearance curve of the subcutaneous tissue cannot be monoexponential: In this tissue an accumulation followed by a washout occurs as evidenced by the radioautographic and tissue dissection studies. This clearly shows that a more detailed analysis of the exchange processes between the two tissues must be made before a resolution of the composite clearance curve (Fig. 3) is attempted.

**Figure 8**

*Clearance curve from subcutaneous tissue alone after labeling with gaseous 133Xenon for 1 minute.*
Transport from cutaneous to subcutaneous tissue is effected by diffusion across the boundary of the two tissues. However, the main transport mechanism seems to be diffusion of $^{133}$Xenon out of cutaneous venous blood flowing through the subcutaneous tissue (transport by both convection and diffusion). This was concluded from the above mentioned autographic and tissue dissection studies comparing subcutaneous deposition with and without maintenance of blood flow. It was calculated from the microdissection studies that 1 hour after the injection the ratio between the amount transported by blood and that transported by diffusion alone was 4:1. But the sources of error of this method will tend to give an underestimation of the ratio which therefore should be higher, perhaps 5:1 or 6:1. These ratios are roughly in agreement with a subjective evaluation of the radioautograms.

The magnitude of the transport of $^{133}$Xenon from subcutaneous back to cutaneous tissue was evaluated from experiments with another radioactive inert gas, $^{85}$Krypton. This isotope emits mainly $\beta$-particles, which can be registered by external monitoring with a Geiger-Müller tube. Because of the selfabsorption of the $\beta$-particles, which have a maximum energy of 0.67 Mev (half-thickness value 0.25 mm), only the radiation from the superficial 1 to 2 mm of the tissue will be counted by this technique (13). The radiation measured will thus in practice be emitted from the cutaneous tissue which is about 2 mm thick. A depot of $^{85}$Krypton in saline was injected superficially in the subcutaneous tissue just underneath the cutaneous layer. A depot of $^{85}$Krypton in saline was injected superficially in the subcutaneous tissue just underneath the cutaneous layer. The injection needle was introduced through the subcutaneous tissue for a distance of 15 cm and after the injection the needle canal was screened with lead. This procedure was used to avoid counting of a reflux of $^{85}$Krypton to the cutaneous tissue via the needle canal.

Immediately after the injection the count rate was that of the background. The count rate increased to a maximum in about 60 minutes (average 63 minutes, so 11, in four experiments). The maximum counting value was about 1% of that the same amount of $^{85}$Krypton could yield when it was injected intracutaneously. For $^{133}$Xenon, which is almost twice as soluble in fat as krypton, the corresponding figure should be approximately 2%. Furthermore, as only about 50% of a cutaneous depot reaches the subcutaneous tissue, the redistribution of $^{133}$Xenon should be only about 3% of the original cutaneous depot.

In the experiments previously referred to, where the distal 2 mm of the skin fold between the thumb and the forefinger was used, or where a skin fold was raised on the back of the hand, the transport from the subcutaneous to the cutaneous tissue was also found to be completely negligible as there was no slow tail of the clearance curves.

Calculation of Cutaneous Blood Flow: the Model. — $^{133}$Xenon deposited in the cutaneous tissue does not remain localized to this tissue during the washout process as the gas accumulated in the subcutaneous fat mainly because of diffusion through the thin-walled veins draining labeled blood from the cutaneous tissue. If this diffusion process reaches equilibrium, then the two tissues function strictly in series with respect to $^{133}$Xenon clearance. Assuming both tissues have a monoexponential frequency function of tracer transit times, then an in-series model predicts a combined clearance curve which should be convex upwards (as seen from equation 5 in the appendix for the case $E = 1$). However, since all curves studied were concave upwards, a more complex model had to be used.

The model here proposed is a combined in-series and in-parallel model. It assumes that under given steady state conditions a constant fraction ($E$) of cutaneous venous blood is extracted from $^{133}$Xenon as it passes through the subcutaneous tissue, due to a more than ten-fold higher solubility of $^{133}$Xenon in this tissue than in blood.

The model is otherwise based on the same assumptions as used for calculating blood flow in a single homogeneous tissue, viz., diffusion equilibrium between tissue and venous blood,
and a knowledge of the tissue to blood partition coefficient.

These assumptions will, as described in the appendix, lead to the following expression for the combined clearance curve

$$Q(t) = \left(1 - \frac{E \cdot k_c}{k_c - k_s} \cdot e^{-k_c \cdot t}\right) + \left(\frac{E \cdot k_c}{k_c - k_s} \cdot e^{-k_s \cdot t}\right),$$

where $k_c$ and $k_s$, the rate constants of the two exponentials, are the blood flow-partition coefficient ratios.

From this it follows that cutaneous blood flow, $f$, expressed per 100 g of tissue, is

$$f = \frac{\ln 2 \cdot \lambda \cdot 100}{T_{1/2}},$$

where $\ln 2$ is the natural logarithm of 2, $\lambda$ is the tissue to blood partition coefficient in ml/g, and $T_{1/2}$ is the half-time of the faster component of the conventional biexponential resolution of the clearance curve.

For cutaneous tissue a $\lambda$ value of 0.7 ml/g is used. This value is calculated from the known composition of the cutaneous tissue with respect to water, protein, and fat (14, 15). For subcutaneous tissue a $\lambda$ value of 10 ml/g is used (12). Values obtained for subcutaneous tissue are presented in Table 1. They are in good agreement with previous findings.

The ratio of the average value of the intercepts of the curves in the experiments presented in Table 1 was 0.48:0.52, SD 0.17 (10 experiments) (see Figures 5 and 9). From this ratio and from the rate constants, the extracted fraction $E$ was calculated to be 0.50. This suggests that about half of the $^{133}$Xenon in the effluent venous blood from the cutaneous tissue leaves the blood and accumulates in the subcutaneous tissue.

Blood Flow in Cutaneous Tissue in Man.—The epicutaneous $^{133}$Xenon application technique was used on the lateral region of the leg in ten normal subjects. The subjects were relaxed and normally dressed, except for the region to be measured, and placed in a recumbent position at an ambient temperature of 19° to 22°C and a relative humidity of about 60%, i.e., non-sweating conditions. Skin temperature was about 32° to 33°C in the area measured. Clearance curves were always fitted readily by two exponentials (Fig. 9). The cutaneous blood flow averaged 5.7 (SD 1.3) ml/100 g • min (see Table 1).

Simultaneous microinjections of $^{133}$Xenon were performed intracutaneously in the identical region on the opposite leg (0.003 ml of $^{133}$Xenon in saline, injected in 3 minutes with a needle of 0.25 mm outer diameter). The initial slope of the injection curves was steeper than that of the curves obtained after epicutaneous application, indicating a hyperemia effected by a trauma of injection. This effect lasted 15 to 20 minutes. Graphic resolution of the injection curves after 20 minutes was found to give a monoeponential fast component, average value 6.1 (SD 1.2) ml/100 g • min (see Table 1).

In Table 2 results are also listed for some other regions: forearm, abdomen and cheek (in regions with and without active hair growth). The results demonstrate considerable regional variations.

A few experiments have been performed on vasoconstricted cold skin of the leg with the epicutaneous labeling technique. In these experiments the fast (cutaneous) component failed to appear. After 10 to 15 minutes of ischemia, hyperemia occurred and the blood flow calculated from the fast component was about 30 ml/100 g • min.

Discussion

$^{133}$Xenon Clearance Method for Measurement of Cutaneous Blood Flow.—The epicutaneous $^{133}$Xenon clearance method described in this paper is indirect. It is based on a two-compartment model which may not, of course, be rigorously correct. Development of the model was based on observations that the clearance curves from the cutaneous and the subcutaneous tissues separately are both practically monoeponential. However, to resolve the curve into two well defined components the following conditions must be fulfilled: The clearance rate of the tail of the curve (the
Clearance curve after epicutaneous labeling with gaseous $^{133}\text{Xe}$ for 3 minutes. Solid circles depict the measured washout curve and open circles the result of a graphic resolution. The mathematical expressions of the two exponentials obtained by the graphic resolution are found in equation 5 in the appendix. The separate washout curve for the cutaneous tissue is constructed by drawing a line parallel to a straight line through the open circles from the top of the measured curve. By subtracting the values given by this line from the measured values the separate curve for the subcutaneous tissue is constructed, denoted by crosses. The mathematical expressions of these separate curves for the two tissues are found in equation 4 in the appendix.

Finally, slope) shall be recorded long enough to be defined adequately; there must be a sufficient difference between the clearance constants of the two exponentials; the difference shall ordinarily be about a factor of 5 or more to give reasonable accuracy (16).

**TABLE 2**

Cutaneous Blood Flow Values Obtained with Epicutaneous Application and with Microinjection Techniques in Various Regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Epicutaneous application</th>
<th>Injection Technique</th>
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<tbody>
<tr>
<td></td>
<td>No. of measurements</td>
<td>Cutaneous blood flow (ml/100 g • min)</td>
</tr>
<tr>
<td>Leg</td>
<td>12</td>
<td>5.7 ± 1.2 (SD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Forearm</td>
<td>1</td>
<td>4.9</td>
</tr>
<tr>
<td>Abdomen</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>Cheek (without hair growth)</td>
<td>1</td>
<td>9.0</td>
</tr>
<tr>
<td>Cheek (with hair growth)</td>
<td>3</td>
<td>18.0</td>
</tr>
</tbody>
</table>

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Furthermore, the intercept on the ordinate of the fast component must not be too small in comparison with that of the slow component. All these conditions seem to be fulfilled in the case of moderately vasodilated skin. The difference in clearance rates was about twenty-five fold, and the ratio of the intercepts (the amounts at the time of the end of the labeling) was about 1:1. This analysis would not be valid when the cutaneous blood flow becomes equal to or less than 1 ml/100 g • min, since the ratio of the fast to slow clearance rates would be less than fivefold, and the intercept of the fast component would be decreased to relatively low values. The 133xenon clearance curves from cold vasoconstricted skin showed slow and practically constant clearance rates immediately after the labeling. The washout model will in this case approach that of a purely in-series model where all 133xenon in the cutaneous blood reaches the subcutaneous tissue, i.e., the fraction $E$ in the biexponential equation approaches unity. That cutaneous and subcutaneous tissue can constitute an in-series arrangement with cutaneous vasoconstriction has previously been observed in experiments with 86 krypton (17).

Arteriovenous shunts do not occur in the regions employed in this study: these are located in fingers, palm, toes, and sole (18). Whether the clearance of 133xenon from the cutaneous tissue in these regions is influenced by the blood flow in the arteriovenous shunts cannot be answered from the results of the present study.

Other sources of error are the clearance of 133xenon from the skin surface by diffusion and the clearance of 133xenon by sweat secretion, both of which have been described in some detail elsewhere (11). It was found that the loss of 128xenon by diffusion from the skin surface only amounted to about 1% to 2% of that cleared by the blood under normal conditions. When the epithelium was stripped off with adhesive plaster, an increase of the loss by a factor of 30 to 40 was found. The middle one-third of the epithelium was found to constitute a diffusion barrier to 133xenon. Under normal conditions the loss from intact skin surface by diffusion would be negligible in the present blood flow studies. But, when the relative gastight epidermal membrane is lost as in burns and some skin diseases, then the method will be invalidated. However, in these cases the epidermal membrane can be imitated by a thin gastight Mylar membrane made to adhere to the skin surface by a drop of water. The loss of 133xenon by profuse sweat secretion was found to amount to as much as 10% to 25% of that cleared by the blood flow and can thus be a significant source of error. The insensible sweat secretion produced under nonsweating conditions will be incorporated in the loss by diffusion from the surface of the skin and is thus negligible.

Under normal conditions these problems are not significant. However, the validity of the use of the exponential washout model on the cutaneous tissue depends on the condition that diffusion equilibrium between the tissue and its effluent venous blood is maintained. That this condition is fulfilled is based on the following:

1. From a theoretical point of view diffusion equilibrium between the tissue and the effluent venous blood must exist. Calculations of the time required for equilibrium by diffusion, taking into account the actual distances, show that this time is essentially shorter than the time taken for the blood to pass through the capillaries (19). Furthermore, the monoexponential shape of the washout curve from the cutaneous and also from the subcutaneous tissue suggests that equilibrium must be maintained during the washout process. Any form of disequilibrium caused by a diffusion limitation will result in a continuum of clearance rates causing a bending of the washout curves (20).

2. The existence of equilibrium for 133xenon between the tissue and the effluent venous blood is also supported by the agreement found in other tissues between the 133xenon clearance method and the directly measured blood flow. In experiments on the isolated gastrocnemius muscle in cats using labeling by diffusion or saturation by intra-arterial infu-
sion for a long time at a constant rate, the results indicate that a very high degree of equilibrium is maintained during the washout of a large fraction of the tracer (21). Similar comparative studies have been performed on fat pads in rabbits (personal communication, S. Levin Nielsen). Also in these studies agreement was found between directly recorded venous outflow and the blood flow calculated from the slope of the 133 xenon washout curve which remains constant throughout the washout after labeling by diffusion from the surface.

Other Methods Used for Estimating Blood Flow in Cutaneous Tissue.—The heat clearance method described by Hensel (1) has been used on the surface of the skin to estimate blood flow qualitatively, and it has always been considered incapable of yielding absolute values of the cutaneous blood flow. Heat has a diffusibility about 100 times greater than xenon (22). From the results of the present study it is therefore obvious that a heat clearance method used on the skin will yield results which are influenced by the clearance in both the cutaneous and the subcutaneous tissue.

Hardy and Soderstrom (2) estimated the peripheral blood flow at an environmental temperature of 23° to 28°C to be on the order of 0.015 ml/cm² • min using heat loss measurements from the body surface in man. In this heat clearance method which is fundamentally of the same nature as that of Hensel, the peripheral blood flow is for the above mentioned reasons a sum of the cutaneous and partially of the subcutaneous blood flow. A quantitative evaluation of the cutaneous blood flow alone is therefore impossible, but it is likely that it is on the order of 2 to 10 ml/100 g • min from the given data. A similar result was obtained by Stewart and Evans using the same method (3).

Helium uptake from the skin on the extremities and the trunk was studied by Behnke and Willmon (4). At an ambient temperature of 23° to 28°C an average value of peripheral blood flow of 75 ml/m² body surface • min can be obtained from their data (average of seven determinations). As the thickness of the cutaneous tissue averages about 1.7 to 2.0 mm (23), then the corresponding figure per 100 g is about 3 to 4 ml/100 g • min. The existence of a diffusion barrier to 133 xenon in the epidermis makes it very likely that there also is some resistance to diffusion of helium through the epidermis. The effect of such a diffusion resistance will be that the method underestimates the cutaneous blood flow.

The plethysmographic technique only estimates cutaneous blood flow in a semiquantitative or qualitative fashion as complicated subtraction procedures must be applied to correct for blood flow in the subcutaneous tissue and muscle. Plethysmography before and after iontophoresis of adrenalin into the skin has been used for this calculation (5, 6). However, it has been demonstrated that with the iontophoresis employed, blood flow was abolished in both the cutaneous and the subcutaneous tissue (6). This technique is therefore not able to give precise values of the cutaneous blood flow alone. From estimated data for the ratio of the weight of the cutaneous and the subcutaneous tissue and the blood flow of the latter tissue, a cutaneous blood flow on the order of 6 to 9 ml/100 g • min is obtained (17).

Clearance methods based on the use of radioactive tracers have previously been tried for estimating cutaneous blood flow in man (24). It was concluded from a study in which 24 Na was injected intracutaneously that the clearance rate was limited by the capillary permeability and not by the blood flow. This is similar to the conclusion of a study with 24 Na in skeletal muscle (25).

An attempt was made by the present author to use the intra-arterial 85 krypton β-particle clearance method (13) for measurement of cutaneous blood flow in man in a previous study (17). It was concluded that this method was invalidated by diffusion processes, the significance of which were grossly enhanced by the self-absorption of the β-particles in the tissue.

The other methods summarized above have
only allowed rough estimation of cutaneous blood flow. The epicutaneous $^{133}$Xenon clearance method may be the only method hitherto used which can give quantitative values of blood flow in cutaneous tissue.

**Appendix**

The model to be described is composed of two homogeneous compartments symbolized by $C$ for the cutaneous and $S$ for subcutaneous compartment. The input in the system is in the form of an impulse into $C$. The output from $C$ is partly in series, a fraction $E$ of the output reaching compartment $S$, and partly in parallel with $S$ (see Fig. 10).

If an initial amount of one unit of tracer is contained in compartment $C$ and the cumulative output from $C$ at time $t$ is called $H_t$, then the amount retained in compartment $C$ at time $t$, $R_c$, can be written $R_c = 1 - H_t$. With the assumption that compartment $C$ is a well mixed compartment from which there is an exponential washout, the expression

$$R_c = 1 - H_t = e^{-k_c t}$$  \hspace{1cm} (1)

can be written for compartment $C$, where $k_c$ is the rate constant of $C$.

In compartment $S$ there is no tracer at time zero. The rate of tracer input to $S$ is a constant fraction, $E$, of the output rate from $C$, which is obtained by differentiation of $H_t$ and changing the sign. Thus the input rate to $S$, called $I$, is

$$I = E \cdot k_c \cdot e^{-k_c t}$$  \hspace{1cm} (2)

For compartment $S$ it is also assumed that a monoeponential function describes the output rate, i.e., this rate is $k_s \cdot e^{-k_s t}$ if one unit of tracer had suddenly been deposited by an impulse input into $S$. Corresponding to the input rate given by equation 2, the output $O$ from $S$ is therefore the convolution of the impulse response and the actual input rate, $I$:

$$O = \int_0^t E \cdot k_s \cdot e^{-k_s \cdot (t - \tau)} d\tau.$$  \hspace{1cm} (3)

An expression for the retained amount of radioactivity in $C$ and $S$ combined, $R_c$ plus $R_s$, called $Q(t)$, can now be calculated inserting equations 1, 2, and 3:

$$Q(t) = R_c + R_s$$

$$Q(t) = R_c + \int_0^t I dt - \int_0^t O dt$$

$$Q(t) = e^{-k_c t} \cdot \left[ \frac{E \cdot k_c}{k_c - k_s} \cdot (e^{-k_s t} - e^{-k_c t}) \right]$$  \hspace{1cm} (4)

$$Q(t) = \left[ \frac{(1 - E \cdot k_s)}{k_c - k_s} \cdot e^{-k_s t} \right] + \left[ \frac{E \cdot k_s}{k_c - k_s} \cdot e^{-k_c t} \right].$$  \hspace{1cm} (5)

The first term in equation 4 is the residual radioactivity in cutaneous tissue $R_c$. A graphic resolution of the observed curve is given by equation 5. This curve resolution will thus give the clearance rate for the cutaneous and the subcutaneous tissue. Since $k_c$ and $k_s$ are observed experimentally the fraction extracted, $E$, can be calculated from the intercepts.

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