A new method for noninvasive measurement of cutaneous blood flow is laser-Doppler flowmetry. The technique is based on the fact that laser light is back-scattered from the moving red blood cells, with Doppler-shifted frequencies; these impulses lead to photodetectors and are converted to flow signals. In this work we used a new system with a low noise level. Comparison was made between this technique and the atraumatic epicutaneous 133Xenon technique for measurement of cutaneous blood flow during reactive hyperemia and orthostatic pressure changes. The laser-Doppler flowmeter seems to measure blood flow in capillaries as well as in arteriovenous anastomoses, while the 133Xe method probably measures only capillary flow. A calibration of the laser-Doppler method against the 133Xe method would appear to be impossible in skin areas where arteriovenous anastomoses are present. The changes in blood flow during reactive hyperemia, orthostatic pressure changes, and venous stasis were found to be parallel as measured by the two methods in skin areas without shunt vessels. The laser-Doppler flowmeter would appear to be a useful supplement to the 133Xe washout method in cutaneous vascular physiology, but it is important to keep in mind that different parameters may be measured.

A new noninvasive method for measurement of cutaneous blood flow is the laser-Doppler flowmeter [1-7]. Monochromatic light from a laser is led by an optical fiber to a skin area, which is permeated by the light to a limited depth. According to the principle of Doppler, the laser light is back-scattered with shifted frequencies when reflected from moving elements such as red blood cells. When reflected from static structures, the frequencies are not shifted. Because of the complexity of the microvascular network, the red blood cells have different velocities and the laser beam reaches the cells at varying angles. As a result, the original laser light is reflected in a broad spectrum of varying frequencies. In the techniques described earlier, systems were used where parts of the reflected light, both shifted and nonshifted, were beating together on the surface of a photodetector. With this method, the noise level was high. In our experiment, therefore, we used a new technique with which the noise level possibly could be reduced significantly [2].

To find a closer relationship between the microcirculation in vivo and the Doppler signal, it was necessary to compare the method with other methods measuring cutaneous blood flow. Holloway and Watkins [1] and Stern et al [5] compared the laser-Doppler method with the washout of intracutaneously injected 133Xe on the forearm. These methods, however, cannot be compared directly because (a) the skin was traumatized by the injection of the tracer, resulting in a local vasodilatation with increased blood flow [8-11], and (b) the laser-Doppler system measures only to a limited depth in the skin, while the 133Xe washout takes place in both the cutis and subcutis. In the following experiments we compared values obtained with the laser-Doppler flowmeter with those obtained with the atraumatic epicutaneous 133Xe application technique. This latter technique eliminated traumatic vasodilatation, permitting direct comparison of the two methods.

MATERIALS AND METHODS
The experiments were performed in 4 normal persons, aged 39 to 60, who adjusted to room temperature at 23°C for at least 30 min before the measurements were started. Informed consent was obtained.
A new laser-Doppler system (Periflux, Perimed, Sweden) was used [2]. The light from a laser is led by an optical fiber to the skin surface. It permeates the skin to a depth of about 1 mm and over an area of about 4 mm². The light is back-scattered with Doppler-shifted frequencies from moving blood cells and with unshifted laser frequencies from stationary tissues. The new system collects part of the reflected light using two optical fibers instead of one and processes it in two separate channels containing photodetectors and circuits for filtration and normalization. The part of the signals that originates from unchanged laser light, including the laser noise as well as some external noise source, is identical in phase and frequency in the two channels; it can, therefore, be suppressed. On the other hand, the Doppler-shifted parts are not equal because they stem from different, though adjacent, microvascular regions. By this method the signal to noise ratio is increased. The flux is normalized square root mean value of the frequency spectrum, which appears at the instrument panel as units of volts.

In the experiments a pen recorder was connected to the output and was adjusted to zero after pointing the Doppler probe toward a white reflecting background. The probe thermostat was adjusted to 32°C, and the instrument was adjusted to a bandwidth at 4 kHz and gain at 10. The filter was set at a high value in order to smooth out fluctuations due to the cardiac cycle.

Vascular Reactions

Reactive hyperemia: A skin area 1 cm in diameter on the fingertip (pulp) of the third left finger was labeled with ¹³³Xe gas by the atraumatic epicutaneous technique described by Sejrsen [10] and Kristensen [11]. The detector was placed 5 cm above the radioactive field. Pulses were fed into a y-spectrometer with a window set around the 81 keV peak of ¹³³Xe. The activity was recorded in intervals of 10 sec. The arm was immobilized in a vacuum cushion at heart level and a miniature blood pressure cuff was placed on the finger proximal to the ¹³³Xe depot. Immediately after the radioactive labeling procedure, measurements of the undisturbed tracer washout were carried out. The cuff was then inflated to 300 mm Hg for 6 min, after which it was deflated and the washout of tracer was followed for about 15 min until the resting value was reached. The count values were corrected for background activity and plotted in a semilogarithmic diagram vs. time.

Blood flow during reactive hyperemia was measured by the laser-Doppler technique in the fingertip as described above and also in the skin fold between the first and second fingers of the left hand. In the case of hand skin, the blood pressure cuff was placed on the upper arm. The probe was placed on the volar side of the skin fold approximately 2 mm from the edge and was fixed with adhesive tape.

Control experiments: Further experiments were undertaken in 1 normal person. A skin area 1 cm in diameter between the first and second fingers on the left hand was labeled with ¹³³Xe gas by the atraumatic epicutaneous technique described by Sejrsen [10]. In order to measure the tracer washout in hand skin alone, the hand was covered by a lead shield except for the distal 2 mm of the skin fold. Measurements of tracer washout were performed as described above.

In this subject, cutaneous blood flow and reactive hyperemia were measured in hand skin and finger skin by both methods.

Orthostatic pressure changes: The ¹³³Xe washout rate was measured in the skin fold with the hand at heart level [1], lowered 40 cm [1], and again at heart level [2]. If sufficiently high ¹³³Xe activity made it possible, the sequences were repeated with the same depot. Blood flow changes during the same maneuver were measured by the laser-Doppler technique, the only difference being a lowering of 50 cm for practical reasons.

Venous stasis: With the hand placed at heart level, venous stasis was induced in the arm by inflating the cuff to 40 mm Hg. The blood flow was measured before [1], during [1], and after [2] venous stasis in the skin fold area.

Calculations

A typical ¹³³Xe washout curve during reactive hyperemia is shown in Fig 1. Calculations of ¹³³Xe washout rate constants (k) and repayment under hyperemia have been described [11, 12]. The relative maximum washout rate constant was calculated from the formula: $k_{\text{max}} = k_{(p)} + k_{(phi)}$. Relative blood flow during lowering and venous stasis was calculated by the formula: $f_{(p)}(t) = f_{(phi)}(t) + f_{(0)}$, as previously described [11]. The Doppler values were read directly from the pen recorder. A typical hyperemia experiment is shown in Fig 2. Excess flood flow and repayment was calculated planimetrically.

Statistics

Preischemic and posthyperemic blood flow values, $f_{(p)}$ and $f_{(phi)}$, and relative blood flow obtained by the two methods were compared by means of Student’s t-test for paired samples. A 0.05 limit of significance was chosen. In figures and text, S.E.M. signifies standard error of the mean.

RESULTS AND DISCUSSION

During the experiments, it was observed that blood flow values obtained by the laser-Doppler flowmeter were significantly influenced by auditory noise. As a consequence, great care was taken to create quiet surroundings.

In the skin of fingers and palm, arteriovenous anastomoses exist between arterioles and venules [13]. These are supplied from a subpapillary plexus, which runs in parallel with the skin surface. One can expect that the laser light, after penetrating the layer of keratin (200-400 µm) of the fingertips, will reach the horizontal subpapillary plexus. The keratin layer of the skin fold between the first and second fingers is probably thinner, so that the laser will permeate even deeper into the cutis.

Arteriovenous anastomoses are active in thermoregulation but the blood flow in these specialized vessels does not participate in nutrition of the tissues. The phenomenon of reactive hyperemia in response to vascular occlusion has been investigated in cutaneous tissue in fingers and in subcutaneous tissue [11]. Blood flow increases following vascular occlusion and excess blood flow, i.e., the integrated blood flow during the period of reactive hyperemia minus the integrated preischemic blood flow for a period of the same duration, increases with duration of vascular occlusion [12]. Repayment, i.e., excess cumulative blood flow divided by preischemic blood flow times duration of vascular occlusion is dependent on metabolic factors. In organs where blood flow is metabolically determined, repayment is often more than 100% while in organs where blood subserves other purposes repayment may be less than 100%.
The results of the reactive hyperemia experiments in 4 normal persons are shown in Table I. There were no significant differences between preischemic and posthyperemic blood flow values as measured by the $^{133}$Xe technique or by the laser-Doppler technique, a finding which validates the hyperemia experiments ($p > 0.3$). The resting blood flow value obtained by the $^{133}$Xe method compares well with previous measurements [11,14] as do the hyperemia parameters including a repayment value around 60% [11,12]. The laser-Doppler measurements of fingertip blood flow were significantly different from the $^{133}$Xe values with regard to maximum blood flow and repayment ($p < 0.01$), while the measurements in hand skin compared well with the fingertip $^{133}$Xe values ($p > 0.1$).

By the laser-Doppler technique the finger pulp blood flow values were almost 5 times the hand values. By the $^{133}$Xe technique hand skin blood flow has been previously measured to be only slightly lower than fingertip blood flow [11, 14]. In Table II the results of control experiments in 1 normal male are shown. Relative maximum blood flow and repayment differed significantly in the hand as well as in the finger when the two methods were compared in these experiments, although the repayment value measured by the laser-Doppler technique in the hand was considerably higher than that measured in the finger. $^{133}$Xe measurements, however, show very similar results in hand and in finger. The resting blood flow values obtained in the finger pulp were only 16% higher than the hand values when measured by the $^{133}$Xe technique but 130% higher when the laser-Doppler technique is used.

The arteriovenous anastomoses subserving temperature regulation can explain why the blood flow values as measured by the laser-Doppler technique are higher in the fingers than in the hand skin fold. The difference between hand and finger values obtained by the $^{133}$Xe and the Doppler method could be explained if the $^{133}$Xe method had measured only capillary blood flow, while the Doppler method was able to measure at least part of the blood flow in arteriovenous anastomoses. Further support for this difference was obtained in the reactive hyperemia response, as only nutritional blood flow needs to be repayed. In the present investigations, regions with and without arteriovenous anastomoses have been examined by the $^{133}$Xe washout method. The question as to whether blood that flows through such shunt vessels becomes equilibrated with the tissue was not investigated previously [10]. The exchange conditions between the blood in these vessels and the tissue are essentially of the same nature as those found for arterioles and venules; it is therefore possible to imagine that by means of the $^{133}$Xe method at least part of the blood flow in the shunt vessels is measured. The present results taken together with previous measurements of cutaneous blood flow in hand skin [14] seem to indicate that only a small fraction of shunt blood flow is measured by the $^{133}$Xe method.

One to 1.5 min after the arterial occlusion, the flow values decreased to 0.1 (V) in the hand and 0.13 (V) in the finger pulp as measured by the Doppler method. These values were constant during the ischemia period (Fig 2). The blood flow value is not zero during ischemia, for one of the following reasons: (1) After the occlusion there might still be a redistribution of red blood cells in the capillaries; however, this would gradually decline during the 6 min. (2) Noise from the laser. The explanation might be a combination of (1) plus (2). The error amounted to 8% in the fingertips and 15% in the skin fold. This amount of noise was subtracted from the "flow values."

The blood flow values obtained by the $^{133}$Xe method deviated

### Table I. Blood flow values obtained by the $^{133}$Xe and laser-Doppler methods in reactive hyperemia experiments in four normal persons (each person was investigated twice on separate occasions)

<table>
<thead>
<tr>
<th></th>
<th>Fingertips (N = 8)</th>
<th>Hand (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{133}$Xe method</td>
<td>Laser-Doppler</td>
</tr>
<tr>
<td>Preischemic blood flow</td>
<td>10.1 ± 0.8 ml/100 g·min$^{-1}$</td>
<td>1.7 ± 0.15 min</td>
</tr>
<tr>
<td>Maximum blood flow</td>
<td>73.9 ± 4.3 ml/100 g·min$^{-1}$</td>
<td>2.1 ± 0.11 (V)</td>
</tr>
<tr>
<td>Relative maximum blood flow</td>
<td>7.6 ± 0.7</td>
<td>1.5 ± 0.04</td>
</tr>
<tr>
<td>Hyperemia duration</td>
<td>1.2 ± 0.1 min</td>
<td>1.7 ± 0.15 min</td>
</tr>
<tr>
<td>Repayment</td>
<td>59.8 ± 4.4%</td>
<td>7.7 ± 1.80%</td>
</tr>
</tbody>
</table>

"N = number of observations in a single patient.

### Table II. Blood flow values obtained by the $^{133}$Xe and laser-Doppler methods in reactive hyperemia experiments

<table>
<thead>
<tr>
<th></th>
<th>Hand (N = 7)</th>
<th>Finger (n = 5)</th>
<th>Hand (N = 10)</th>
<th>Finger (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemic blood flow</td>
<td>8.60 ± 0.7</td>
<td>9.70 ± 0.6</td>
<td>0.71 ± 0.09</td>
<td>1.68 ± 0.11</td>
</tr>
<tr>
<td>Posthyperemic blood flow</td>
<td>8.00 ± 0.4</td>
<td>9.60 ± 0.8</td>
<td>0.73 ± 0.07</td>
<td>1.81 ± 0.13</td>
</tr>
<tr>
<td>Maximum blood flow</td>
<td>44.10 ± 3.5</td>
<td>77.30 ± 3.4</td>
<td>2.00 ± 0.1</td>
<td>2.38 ± 0.08</td>
</tr>
<tr>
<td>Relative maximum blood flow</td>
<td>5.56</td>
<td>8</td>
<td>2.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Duration of hyperemia</td>
<td>1.74 ± 0.08 min</td>
<td>0.52 ± 0.03 min</td>
<td>3.60 ± 0.2 min</td>
<td>2.10 ± 0.07 min</td>
</tr>
<tr>
<td>Repayment</td>
<td>71.90 ± 9.8%</td>
<td>61.40 ± 4.7%</td>
<td>33.00 ± 5.2%</td>
<td>3.60 ± 0.7%</td>
</tr>
</tbody>
</table>

"N = number of observations in a single patient.

![Fig 3. Repayment values obtained by the $^{133}$Xe and the laser-Doppler methods, plotted against preocclusive blood flow, in one subject.](image-url)
from zero during vascular occlusion due to diffusion of $\text{Xe}$ gas to the atmosphere and secretion of $\text{Xe}$ by sweat [10]. In fingertips the “washout” during vascular occlusion amounted to 25% but was less than 10% in the skin fold. The $\text{Xe}$ blood flow values were corrected for this error as previously described [11].

As only nutritional blood flow needs to be repayed, these results add further support to the conclusion that the laser-Doppler method measures at least part of the blood flow in arteriovenous anastomoses. As measured by the $\text{Xe}$ method, repayment in hand and in finger was equal, as one would expect when only nutritional blood flow is measured.

The repayment values from the control experiments are plotted vs. the preocclusive blood flow in Fig 3. The figure shows a negative correlation between repayment and preocclusive blood flow values in both methods. The correlation between preocclusive blood flow values and repayment in the hand seems, for both methods, to be a double logarithmic function. By the $\text{Xe}$ method, the coefficient of regression was calculated to be 0.65 and for the Doppler method 0.83 ($p < 0.05$).

In the experiments when the hand was lowered below heart level, we found a relative blood flow of 0.78 ± 0.05 S.E.M. for the hand when using the $\text{Xe}$ method and 0.6 ± 0.04 S.E.M. when using the Doppler method. The relative values did not differ significantly in the two methods ($p < 0.05$), and the value differed significantly from 1 (Fig 4).

The decrease in blood flow during lowering is due to an increase in vascular resistance (a vasoconstrictor response). This response is evoked when vascular transmural pressure is elevated 25 mm Hg or more [15]. Lowering of the arm 40 cm below heart level corresponds to an increase in vascular transmural pressure of about 30 mm Hg. The same constrictor response was evoked when venous stasis at 40 mm Hg was induced in the arm. When the $\text{Xe}$ method was used, the relative blood flow was 0.7 ± 0.03 S.E.M., and by the Doppler method 0.69 ± 0.03 S.E.M. These values do not differ significantly.

From these results it may be concluded that the laser-Doppler method is able to measure relative cutaneous blood flow changes during hyperemia and orthostatic pressure changes. The arbitrary values obtained by the laser-Doppler method cannot be calibrated against the atraumatic epicutaneous $\text{Xe}$ washout technique in skin areas with shunt vessels.

Our findings indicate that fundamental differences exist in the parameters measured by these two methods. The laser-Doppler method might prove useful in investigations of vascular participation in temperature regulation.

The authors are grateful to Perimed, Stockholm, Sweden, for placing the laser-Doppler instrument at our disposal.

REFERENCES