RADIOACTIVE XENON TISSUE CLEARANCE: STANDARDIZATION FOR MEASUREMENT OF PERIPHERAL BLOOD FLOW

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Investigators have long attempted to measure peripheral blood flow by a wide variety of methods, each of which may have practical or theoretical problems or limitations. Particularly in older patients with occlusive arterial disease, direct-measurement techniques are impractical, especially if repeated measurements must be made. The indirect method using plethysmographs is cumbersome for application where blood flow must be measured during exercise, a condition which is difficult to reproduce and quantitate. Even the Fick principle, when applied to the extremities, requires both arterial and venous punctures and is incapable of providing blood-flow measurements under rapidly changing physiologic conditions. Other indirect techniques have been used, but a major problem encountered is the inability to standardize such methods to obtain more than relative changes in blood flow.

In attempting to develop a more instantaneous and less traumatic technique which could be adapted to humans Kety (*1*) in 1949 used tissue isotope clearance with ^{24}Na as a measure of regional circulatory efficiency and showed changes in the clearance rate constant (k) with ischemia and exercise. With such tissue injections the radioisotope is removed only minimally by diffusion but is almost all cleared by being picked up from capillary blood flowing in an equilibrated state through the tissue. Its removal is, therefore, proportional to arterial capillary flow which equals the sum of venous and lymphatic flow (removal). The actual isotope being removed is proportional to the quantity still remaining, so that an exponential clearance curve was found. After presenting the theoretical background, Kety used ^{24}Na by injection to estimate skeletal muscle-clearance constants and found that these were directly related to cuff occlusion, vasoconstriction, reactive hyperemia and exercise. Kety's results were extended by Walder (*2*) to include patients with arterial disease.

Lassen and others (*3–5*) have compared ^{133}Xe with ^{24}Na as the isotope to be used in such clearance studies. As finally adopted by Lassen and his group (*5*), the counting rate after injection of ^{133}Xe was recorded on a strip chart using a counting-rate meter with a logarithmic potentiometer. Analysis of the data was then carried out graphically. They were able to calculate blood flow (ml/100 gm/min) because they added the factor of muscle-to-blood partition coefficient for xenon. While absolute muscle flow was calculated, the ability to use counting statistics for estimating confidence limits of the measurements was lost because of the graphic recording method.

The following technique was, therefore, developed, standardized and applied to avoid some of the problems encountered by Lassen and his group. Direct digital readout was adopted rather than graphic recording with a counting-rate meter so that appropriate counting statistics could be included. Furthermore, correction of the partition coefficient for variations in hematocrit was introduced. The technique was then applied under various conditions and compared with other clinical and laboratory parameters.

METHODS

Radioactive. The general technique adapted by Lassen and his group was closely followed. A solution of ^{133}Xe in physiologic saline was used with a small dilution volume of 0.2 ml or less containing from 50 to 300 μCi. For muscle study flows, this was injected into the thickest part of the anterior tibial muscle using a sharp needle with an outside diameter of 0.4 mm; this needle was inserted 1.5 cm at

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an angle of 45 deg to the skin surface. Reproducibility of repeated studies was assured by locating the injection site 10 cm distal to the inferior border of the patella and 2 cm lateral to the tibial crest. The 45-deg angle to the surface is directed inferiorly from the injection site. In all cases, the plunger of the syringe was withdrawn before injection to ensure that intravenous injection was not occurring. Subsequently similar tissue-clearance studies were carried out with intracutaneous injections. Such skin-clearance studies may be adapted for the study of patients with vasospastic disease of the upper extremities as well as arteriolar disease in the lower extremities.

Although the gamma radiation from ^{133}Xe is relatively weak, it is readily detected by a NaI(Tl) scintillation probe, which, in these studies, was placed in a constant geometric relationship to the injection site by a plastic probe (9.0 cm from the skin to the tube). The particular scaler and its controls (Baird-Atomic Model 812B) permitted continuous counting at predetermined intervals with immediate reset and automatic strip recording of the counted values. With appropriate shielding, it was possible to study two separate injection sites simultaneously by means of individually placed, separate detectors and scalers.

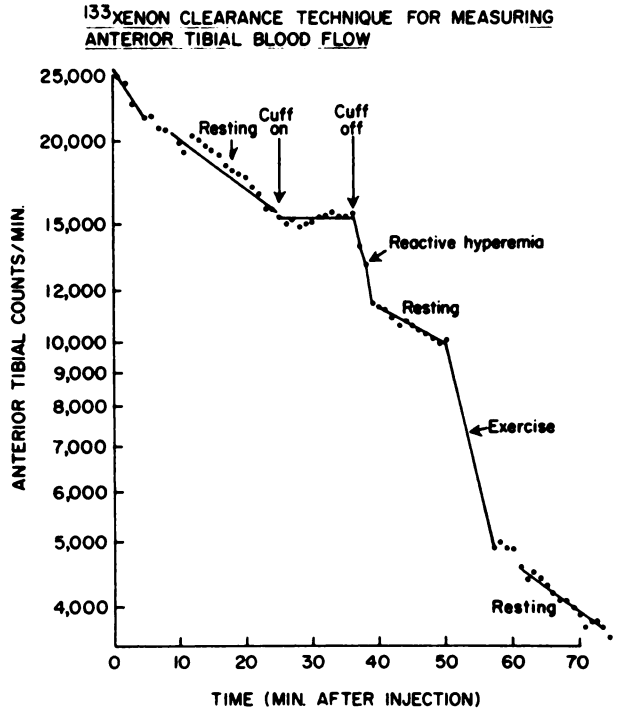

FIG. 1. Semilogarithmic plot of raw data during typical anterior tibial muscle blood-flow study. Flat portion during cuff occlusion and steep slopes after occlusion and during exercise demonstrate sensitivity of method under rapidly changing conditions.

During the initial study standardization period, a number of studies were carried out in accordance with a rigid protocol which included individual 1-min counts at rest for 25 min following the injection. Counts were then continued for an additional 10 min with blood flow occluded by a large thigh pressure cuff inflated to a level exceeding systolic blood pressure. By this means after an initial fast disappearance (3–5 min), a control resting flow clearance rate could be measured and the completeness of occlusion ascertained (Fig. 1).

Additional 1-min counts obtained for the next 15 min showed immediate reactive hyperemia followed by reestablishment of the resting clearance rate (Fig. 1). At 50 min, a standardized treadmill exercise period was interjected which remained identical for each patient during the course of his studies. The total disappearance during this period permitted calculation of the blood flow during exercise, and the subsequent 1-min counts for an additional 20 min or so permitted evaluation of the post-exercise hyperemia and reestablishment of the resting clearance rate.

After appropriate analysis of the data obtained during this standardization phase, it was found that the total test period could be contracted without significant loss of physiologic information. The computer program (see below) which had been developed to handle the original standardized technique was readapted to permit inclusion of similar events with altered time sequences. The contracted test ordinarily lasts 46 min, with an initial resting period of 15 min, with the application of the cuff for 10 min and with only 5 min for study of reactive hyperemia. Exercise is then undertaken, with an additional 5 min following exercise. With this technique one can evaluate resting flow, reactive hyperemia to ischemia, the effects of exercise itself and any post-exercise hyperemia which may be related to an oxygen debt in patients with arteriosclerosis obliterans. For subcutaneous flow studies, occlusion and exercise are normally omitted with continuous 1-min counts being recorded for only 25 min.

Mathematical and computer. The blood-flow calculation carried out followed the formula of Alpert *et al* (*5*) in relating the blood flow to the disappearance rate and the partition coefficient of ^{133}Xe between muscle and blood. Their formula for blood flow is:

$$MBF = -100\,\lambda \ln 10\,D \text{ (ml/100 gm/min)} \quad (1)$$

where MBF is the muscle blood flow, 100 is the multiplication factor required for 100 gm of tissue, λ is the muscle-to-blood partition coefficient and

CONDITION	BLOOD FLOW	STD.ERR.
INITIAL FAST RATE(5)	2.429	-0.261
RESTING (8 TO 25 MINUTES)	1.030	-0.073
CUFF (26 TO 35 MINUTES)	0.430	-0.108
REACTIVE HYPEREMIA MINUTE 1	2.195	-0.796
REACTIVE HYPEREMIA MINUTE 2	1.899	-0.807
RESTING (38 TO 50 MINUTES)	0.333	-0.059
EXERCISE	4.000	-0.223
POST EXERCISE MINUTE 1	2.999	-0.947
POST EXERCISE MINUTE 2	0.042	-0.956
POST EXERCISE MINUTE 3	1.854	-0.962
POST EXERCISE MINUTE 4	0.514	-0.970
POST EXERCISE MINUTE 5	-0.019	-0.972
RESTING REMAINING MINUTES - 75	0.551	-0.033

FIG. 2. Typical computer output showing muscle blood flow and its standard error of estimate as obtained from raw data from study such as that shown in Fig. 1.

D is the slope of the logarithmic disappearance curve as a fraction of a decade per minute. By our computer program, D was calculated by two separate methods depending on whether we were studying the slope over a period of time under stable conditions or whether we were comparing instantaneous flow by using the counting rate of two separate minutes measured sequentially. Whenever multiple points were available (as during rest or during the occlusion period induced by inflation of a blood pressure cuff), the logarithmic decrement (D) was determined by semilogarithmic regression analysis and was expressed as the disappearance half-time for convenience.

Although Alpert *et al* (*5*) used a standard partition coefficient, λ, of 0.7 in their calculations as the partition coefficient of muscle to blood, it is apparent that such a constant partition coefficient is not applicable, particularly in anemic patients. Conn (*6*) did extensive studies of the equilibrium distribution of radioactive xenon in various tissues. He reported that the relative solubilities of xenon were:

in	water	1.00
	plasma	1.45
	erythrocytes	3.75
	skeletal muscle	1.62

Therefore, the partition coefficient between muscle and whole blood is

$$\lambda = \frac{\text{Solubility in muscle}}{\text{Solubility in whole blood}} = \frac{\text{Solubility in muscle}}{\text{Solubility in RBC} + \text{solubility in plasma}} \quad (2)$$

or

$$\lambda = \frac{1.62}{\frac{3.75\ \text{Hct}}{100} + \frac{1.45\ (100 - \text{Hct})}{100}} = \frac{162}{145 + 2.30\ \text{Hct}} \quad (3)$$

in which Hct equals hematocrit.

Since the value D is negative, the blood flow is calculated from Eq. 1 as

$$\text{MBF} = -100\ \frac{162}{145 + 2.30\ \text{Hct}}\ \ln 10\ \text{D}\ (\text{ml}/100\ \text{gm/min}) \quad (4)$$

$$= \frac{-3730.9}{145 + 2.30\ \text{Hct}}\ \text{D}\ (\text{ml}/100\ \text{gm/min}). \quad (5)$$

Under each study condition, the disappearance half-life was determined and reported along with its confidence limits. The confidence limits were based upon the estimate of confidence limits of the slope of the regression, when multiple points were used for calculating the muscle disappearance half-time. When the difference was based upon two single points, the application of Poisson statistics was used, as is standard in radioisotopic counting. The individual confidence limits of each of the counting rates is based upon the square root of the total number of counts recorded in the particular counting period.

In simplifying the actual expression of the differences between the logarithmic values, the logarithmic difference actually comes out equal to the logarithm of the ratio of the lower count to the higher count, so that in operations only one logarithmic value must be obtained instead of two, thereby speeding up the computer program. The original confidence limits were carried along at each step of the calculation in order to ultimately provide the estimation of blood flow under various conditions along with the confidence limits of this flow measurement.

A typical contracted output of blood-flow measurements under the 13 conditions initially studied by the standardized procedure is shown in Fig. 2. Since this particular subject had relatively severe arteriosclerosis, relatively little change in blood flow was observed with exercise and reactive hyperemia. It may be noted that the initial fast rate for the first 5 min indicated somewhat more rapid blood flow than was found between 8 and 25 min, listed as resting blood flow. Essentially complete occlusion is assured by having a blood flow not significantly different from zero during application of the cuff, but the blood flow tripled in the first minute following removal of the cuff. Similarly, a tripling of blood flow occurred subsequently during exercise, and although there may be a trend toward post-exercise hyperemia, the elevations were not significant except for the first minute following exercise. The final resting value is similar to that observed during the rest period before exercise and during the rest period between 8 and 25 min.

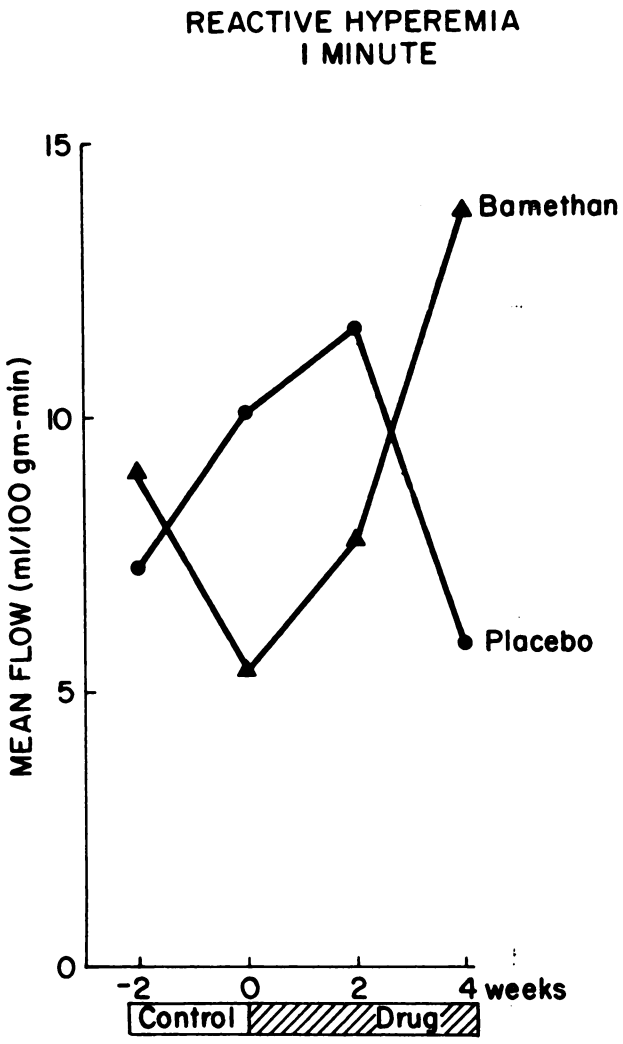

FIG. 3. Mean muscle blood flow changes during first minute of reactive hyperemia after cuff occlusion in 18 patients with arteriosclerosis obliterans treated with placebo or bamethan after baseline observations. (See Table 1.)

Because of the relatively high standard errors found in association with calculating the 1-min values, particularly following exercise as shown in Fig. 2, we customarily provide tabulations which extend over several minutes following exercise or ischemia in order to obtain more valid slope values.

Since the skin flow studies were done without the use of exercise and ischemia, a somewhat different mathematical approach was applied in the analysis of these data. Under these conditions the same lambda is used based upon similar chemical compositions of skin and muscle. With these skin flow studies, a single exponential disappearance rate is not commonly observed. It is possible that the intracutaneous tissue injection site may not be as completely equilibrated with capillary blood as is muscle, so that diffusion could account for the decreasing disappearance rate. Such diffusion may result in xenon being trapped by subcutaneous fat, which has high xenon solubility and which may also account for the progressive decrease in clearance rate. Subcutaneous vascular shunts could also be a factor in the initial fast disappearance rate. In order to improve reliability of the reporting over a period of time, and since a simple semilogarithmic disappearance curve was not found, a technique similar to the running average approach was used with regression analysis determinations of the slope for each consecutive 5-min period reported as the flow at the midpoint of that period. This permitted valid estimates of confidence limits and permitted the comparison of flow rates from one test occasion to another and under varied experimental conditions in the same subject or patient with minimal distortion by experimental variability such as may be induced if only analysis of disappearance between adjacent points is applied.

The radioactive xenon method can be repeatedly carried out using similar techniques in muscle or

skin on several occasions during a single day in order to evaluate the effects of placebo or relatively short-acting drugs on the various parameters of blood flow. Moreover, it can be repeated at weekly or biweekly intervals at a similar time of day following administration of medication on a more subacute basis, and the data obtained (blood flows) can then be readily subjected to rigorous statistical analysis using analysis of variance to isolate the particular effects of drug, time of day and the influence that these have on various parameters of flow studied.

RESULTS

To study the sensitivity of the technique in patients with arteriosclerosis obliterans in which relatively marked impairment of vascular reactivity may be present, 18 such patients were paired by severity of disease, presence or absence of diabetes and age. Half of the patients received placebo and half received bamethan sulfate (100 mg b.i.d.) according to a randomized paired assignment during this treatment period. Following two baseline studies, these patients were restudied after 2 weeks and 4 weeks on active drug or identical placebo. The blood flow was in the relatively normal range in the calf muscles at rest; but during exercise and following an induced ischemia in the same muscles, the blood inflow was far below normal, indicating a decrease in vascular reserve capacity. The mean values during the first

TABLE 1. ANALYSIS OF VARIANCE OF BLOOD FLOW DURING FIRST MINUTE OF REACTIVE HYPEREMIA AFTER CUFF ISCHEMIA

	Source	Degrees of freedom	Mean square
1	Mean	1	5625.2633
2	Drug	1	1.3114 $F(1, 16) < 1$, n.s.
3	Period	1	60.3443 $F(1, 16) = 1.23$, n.s.
4	Session	1	0.3287 $F(1, 16) < 1$, n.s.
5	I (D)	16	147.8998
6	DP	1	52.7382 $F(1, 16) = 1.07$, n.s.
7	DS	1	31.3381 $F(1, 16) < 1$, n.s.
8	PS	1	1.3369 $F(1, 16) < 1$, n.s.
9	IP (D)	16	49.2414
10	IS (D)	16	41.9537
11	DPS	1	376.4448 $F(1, 16) = 5.87$, $P < 0.05$
12	IPS (D)	16	64.1742

TABLE 2. CORRELATION MATRIX BETWEEN FLOWS AND WALKING AND TREADMILL DISTANCES (72 STUDIES)

No* \ No*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00	0.58‡	0.13	0.20	0.16	0.32†	−0.06	−0.06	0.04	0.06	0.04	0.11	0.14	0.34†	0.19
2		1.00	0.15	0.25**	0.45‡	0.47‡	0.22	0.01	0.17	0.13	−0.06	0.08	0.33†	0.28**	0.15
3			1.00	−0.05	−0.12	−0.00	−0.17	−0.03	0.08	0.09	−0.11	−0.00	0.20	−0.25	−0.36
4				1.00	0.12	0.08	0.21	0.31†	0.06	0.08	−0.02	0.21	0.24**	0.45‡	0.38‡
5					1.00	0.27**	0.38‡	0.03	0.09	−0.04	0.08	0.20	0.24**	0.32†	0.31†
6						1.00	0.17	0.07	0.20	0.04	0.26**	−0.08	0.37†	0.29**	0.07
7							1.00	−0.07	0.00	0.01	−0.03	0.05	0.33†	0.19	0.05
8								1.00	0.11	0.26**	0.11	0.14	0.12	0.01	0.12
9									1.00	−0.06	0.07	0.09	0.06	0.09	−0.09
10										1.00	−0.33	−0.27	0.00	−0.17	−0.08
11											1.00	−0.07	0.20	0.15	0.05
12												1.00	0.15	−0.01	−0.03
13													1.00	0.22	0.04
14														1.00	0.72‡
15															1.00

Degrees of freedom = 70
** 0.23, $p < .05$
† 0.30, $p < .01$
‡ 0.38, $p < .001$

* 1. Initial Fast Rate (5)
2. Resting (8 to 25 minutes)
3. Cuff (26 to 35 minutes)
4. Reactive Hyperemia Minute 1
5. Reactive Hyperemia Minute 2
6. Resting (38 to 50 minutes)
7. Exercise
8. Post Exercise Minute 1
9. Post Exercise Minute 2
10. Post Exercise Minute 3
11. Post Exercise Minute 4
12. Post Exercise Minute 5
13. Resting Remaining Minutes to 75
14. Treadmill Distance
15. Walking Distance

minute following ischemia (in other words, during reactive hyperemia) are shown in Fig. 3. This figure illustrates the effect on blood flow of active drug compared to that of placebo. These results will be described elsewhere in more detail for the other flow conditions. It may be seen in Fig. 3 that in the active drug group the muscle blood reserve capacity is improved, with flow reaching the highest value after 4 weeks of therapy. In the group on placebo, although slight improvement appeared after 2 weeks of administration, the mean muscle blood inflow is again decreased after 4 weeks of placebo. These results are shown in the analysis of variance (Table 1) where a significant drug-period-session interaction ($F = 5.87$, $p < 0.05$) is seen.

Table 2 shows a comparison of 13 different flow measurements presented as a correlation matrix for assessing the pertinence of all these blood-flow measurements to standard clinical evaluation, such as walking and treadmill distances. It may be seen that the highest correlation found (0.72) was observed between treadmill distance and walking distance but that good correlations were also observed in the 1- and 2-min reactive hyperemia measurements when compared to both treadmill distance and walking distance. Of additional interest is the negative correlation which occurred between blood flow during the cuff occlusion and the treadmill and walking distances. This may be interpreted as indicating that where flow was relatively severely impaired the arteries were sufficiently sclerotic that occlusion was not complete and flow continued in spite of the occlusion cuff. Although many of the correlations shown at rest were also significant, the magnitude of the increases in blood flow is of doubtful clinical significance. Naturally, since these blood-flow techniques represent new approaches, their validity is therefore independently confirmed by the correlations seen in Table 2.

CONCLUSIONS

In comparing blood-flow measurements following intramuscular radioactive xenon injection under various conditions with standardized treadmill walking distances, correlation was observed among several of the explored parameters. The availability of this technique and its computerization for efficient data handling lets one undertake a more comprehensive investigation under various physiologic and pharmacologic conditions, follow laboratory changes and relate them to the clinical course of the disease as estimated by conventional measures. Wider application of the radioactive xenon technique in clinical and pharmacologic studies is recommended. With the proper computerized support, the technique is practicable for general clinical diagnostic use and is capable of making blood-perfusion measurements which otherwise would require exposure of vessels.

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