Continuous Infusion Indicator Dilution Measurement of Limb Blood Flow and Vascular Response to Magnesium Sulfate in Normotensive and Hypertensive Men

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ABSTRACT A constant infusion, indicator dilution technique for blood flow measurements in the forearm and hand of man was tested and validated in vitro and in vivo. This technique employs jet injection to improve mixing of indicator with arterial blood. The mixing characteristics of the jet injection system were studied in vitro in tubing simulating the brachial artery of man. In addition, actual blood flows in the isolated pump-perfused forelimbs of five dogs were compared with constant infusion, indicator dilution calculated flows. Measurements were also made of mixing and of blood flow in the forearm and hand of man. The technique was used to compare forearm and hand vascular responses with constant intrabrachial arterial infusions of magnesium sulfate in 13 normotensive and 13 essential hypertensive men.

In vitro and in vivo the jet injection system significantly improved mixing of indicator with blood, as compared with mixing produced by standard infusion techniques, without causing hemolysis. In 30 measurements in isolated, perfused dog forelimbs the correlation coefficient between actual and calculated blood flow was 0.992. Resting limb vascular resistance in the hypertensive group was significantly higher than in the normotensive group. Limb vascular resistance in all 26 men decreased in response to intrabrachial-arterial infusion of 0.25% magnesium sulfate (8 ml/min). Rate of infusion of Mg^{++} was 0.162 mEq/min. There was a significant positive linear correlation between level of initial limb vascular resistance and magnitude of response to magnesium sulfate. Vascular response data adjusted for this source of variation were similar in hypertensives and normotensives.

The data suggest that this constant infusion, indicator dilution technique allows accurate calculation of total limb blood flow in man, provided that anomalous bifurcation of the brachial artery is not present. The data also suggest that the jet injection system improves mixing of substances with arterial blood. Thus, use of this system should especially aid reliability of studies of limb vascular responses to vasoactive agents infused into the brachial artery.

INTRODUCTION

For study of normal and abnormal vascular physiology in the limbs of man, measurement of blood flow by an indicator dilution technique such as described by Andres, Zierler, Anderson, Stainsby, Cader, Ghrayyib, and Lilienthal (1) is attractive for several reasons. If there is evidence of good mixing of indicator with limb blood, it may be inferred that an additional substance administered along with the indicator is equally well mixed (2). Such evidence of mixing is important in vascular response and metabolic studies, especially in cases of high bifurcation of the brachial artery, an anomaly occurring in about 20% of humans. In the presence of this anomaly, active substances would be infused into the radial or ulnar artery rather than into the brachial, resulting in inadequate mixing with limb arterial blood and inaccurate response studies.

Second, in contrast to plethysmography, the indicator dilution technique does not interrupt venous outflow from the limb and therefore does not alter the

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pressure gradient nor cause abnormal accumulation of vasoactive metabolites, both of which might change arterial inflow (3). Furthermore, decreases in venous compliance evoked by vasoactive agents do not affect the indicator dilution technique but might decrease arterial inflow during measurements by plethysmography by causing a more rapid rise in venous pressure and, hence, a more rapid decrease in driving pressure gradient. Finally, in the absence of practical techniques for direct measurement of limb blood flow in man, it is desirable to have several independent indirect techniques, each serving as a check on the others.

The major problem associated with accurate indicator dilution measurement of limb blood flow is adequate mixing of indicator with blood. Approaches which have been used to improve mixing include jet injection of indicator to produce turbulence (1) and intrabrachial arterial infusions of extremely large (34 ml/min) volumes of fluid (4). Unfortunately, jet injections which produce turbulence also produce hemolysis and, hence, vasodilation (1). Very large-volume infusions seem undesirable, although reportedly they do not disturb normal limb blood flow, being merely additive (4).

One purpose of the present study was the validation in vivo of the constant infusion indicator dilution measurement of limb blood flow by use of a new type of jet injection system improving mixing by both volume and jet effects. The present study compared actual blood flow with flow calculated by this constant infusion, indicator dilution technique in the isolated, pump-perfused limb of the dog. The technique was also used in man to study anomalous brachial arterial bifurcation and the venous distribution of radial and ulnar arterial blood. Furthermore, the technique was used to measure resting limb blood flow and to study limb vascular responses to intrabrachial arterial infusions of a vasoactive agent, the magnesium ion (5). As there is evidence that responses to vasoactive agents (6) and vascular magnesium metabolism (7) may be abnormal in essential hypertension, vascular responses to the magnesium ion were compared in normotensive and essential hypertensive men. Particular attention was given to the problem of interpretation of such response data in groups with differing levels of initial vascular resistance.

METHODS

Jet injection system

Intrabrachial arterial infusions were made through a 26 gauge stainless steel hypodermic needle modified by Kimray, Inc., Oklahoma City, Okla. Two side holes each of 0.006 inch diameter were drilled into the shank of the needle about 2 mm behind the tip and 180° apart. The needle tip opening was sealed and cut so that the tip was blunt. A Clay-Adams plastic tubing to male Luer lock adapter was soldered to the hub of the needle. A Becton-Dickinson Swinney filter adapter was connected in series with the needle to eliminate any particulate matter in the infusate which might occlude the holes of the needle; plugging of holes was rare. The jet needle was introduced into the brachial artery through a 20 gauge Riley arterial needle (Becton-Dickinson & Co., Rutherford, N. J.), and the adapter on the jet needle hub locked into the hub of the Riley needle.

Because the jet needle required a moderately high volume rate of infusion (8 ml/min) at high infusion pressures (up to 1500 mm Hg), standard infusion pumps proved inadequate. Therefore a new infusion pump, pressure independent to 45 psi and driving two 125 ml stainless steel syringes, was constructed (Kimray, Inc., Oklahoma City, Okla.). Variation of delivery rate of this pump was less than ±1%.

Pressure between the syringe and the jet needle was monitored and provided a check on the patency of the orifices in the jet needle. Kinetic energy of infusate with both holes open was approximately 4500 g cm^2 sec^{-2}, a kinetic energy less than that found by Andres et al. (1) to produce hemolysis in man. The calculated Reynolds number of the infusate from this system is approximately 71, considerably less than that considered necessary to produce turbulent flow in the brachial artery of man (1).

In order to study the mixing characteristics of the jet injection system, IHSA (^{131}I-labeled human serum albumin in isotonic sodium chloride solution, Albumotope, E. R. Squibb & Sons, New York) was infused into citrated human bank blood flowing through a model simulating the human brachial artery and its bifurcation. The model was made of rubber tubing and a polyethylene "Y" tube, each 5 mm I.D., approximately the internal diameter of the brachial artery of man. Adjustable resistances were placed on the two outflow branches so that flow was distributed equally in five experiments and at a ratio of approximately 60:40 in four experiments. Pulsatile blood flows of 25–200 ml/min were provided by a blood pump (Sigmamotor, Inc., Middleport, N. Y.). Pump frequencies were 28–216/min, respectively. These flows are in the range to be found in the brachial artery of man. Standard 20 gauge hypodermic needles and the jet needle were inserted in turn through the wall of the rubber tubing so that the needle tip lay 3 cm upstream to the bifurcation of the tubing. An attempt was made to center each needle in the stream of blood. Infusion rate through the jet needle was 8 ml/min and through the standard needle, 3 or 8 ml/min. Blood outflowing from the two branches was simultaneously collected for measurement of indicator concentrations during the infusions. The difference between indicator concentrations in the two sampled branches was considered an indication of mixing and was expressed, as by Andres et al. (1), as per cent relative difference (rd), the per cent by which a concentration of indicator in either branch differs from their mean concentration: $\mathrm{rd} = \frac{C_2 - C_1}{C_1 + C_2} 100$, where C_1 represents a concentration of indicator in the one branch, and C_2 represents a concentration of indicator in the other branch occurring simultaneously with C_1. Mean relative difference (mrd) represents $\frac{\Sigma \mathrm{rd}}{n}$, the mean of the rd's at a single flow setting.

The mechanism of the mixing produced by the jet needle was also studied by infusing Evan's blue dye through the jet and standard needles into a stream of water flowing through glass tubing (I.D. 5 mm) simulating the human brachial artery. Infusion rate through the jet needle was 8 ml/min, and through the standard needle, 3 or 8 ml/min. The water was pumped by a Sigmamotor pump at pulsatile

flows of between 25 and 200 ml/min. Mixing of dye and water was recorded by high-speed flash photography.

Experiments in dogs

Hemolysis. In order to determine if the jet needle infusate produced hemolysis and vasodilation, a pressure-independent Sigmamotor blood pump was interposed between the femoral artery and brachial artery of mongrel dogs anesthetized with sodium pentobarbital, 30 mg/kg, and given heparin, 10,000 USP units. Blood flow in the brachial artery was thereby held constant, and monitored perfusion pressure measured limb vascular resistance. A standard 20 gauge hypodermic needle and the jet needle were separately introduced into the tubing downstream from the pump. Isotonic sodium chloride solution was infused constantly through each needle at 8 ml/min, and resulting changes in perfusion pressure were recorded on a Sanborn oscillographic recording machine. Perfusion pressure responses were also observed to injections of isotonic sodium chloride or autologous blood through a 27 gauge hypodermic needle at velocities which hemolyzed the injected blood.

Indicator-dilution measurements of blood flow in the isolated, pump-perfused dog forelimb. In order to validate the constant infusion indicator-dilution measurement of limb blood flow using the jet injection system, flow was calculated by indicator dilution and compared with actual blood flow using the technique diagrammed in Fig. 1. Five mongrel dogs (20–30 kg) were anesthetized with sodium pentobarbital, 30 mg/kg, and given heparin, 10,000 USP units. The soft tissue of the forelimbs was totally severed from the bodies, and tourniquets were applied to tissue of both stumps. In four of the five dogs the humerus was also severed. A pressure-independent Sigmamotor pulsatile blood pump was interposed between the femoral artery and brachial artery so that limb blood flow was supplied by pump; in dogs with humerus severed, limb blood flow was supplied solely by pump. Limb venous outflow drained from the cephalic and brachial veins into a reservoir and was measured by timed collection of blood in graduated cylinders; in the four dogs with severed humerus the venous outflow represented total limb blood flow. Pressure was monitored in each vein in order to detect any obstruction to venous outflow; pressures remained less than 10 mm Hg. Blood was pumped from the venous reservoir back into the femoral vein of the animal. IHSA solution was infused at 8 ml/min through the jet injector needle introduced against the direction of blood flow into the pump tubing. In order to limit the distance between site of injection and the bifurcation of the brachial artery, the needle was inserted immediately upstream to the junction between pump tubing and brachial artery approximately 4 cm upstream to the bifurcation. Samples of blood for determination of indicator concentration were simultaneously collected from the two veins and from the tubing upstream to the blood pump (recirculation concentration) at 4 min after beginning each infusion. Venous outflow was measured before and after blood sampling, and flow calculated by indicator dilution was compared with actual venous outflow. These measurements were made at several pump flow settings in each dog. The difference between indicator concentrations in the two sampled veins was considered an indication of mixing and was expressed as per cent relative difference (rd), or as per cent mean relative difference (mrd).

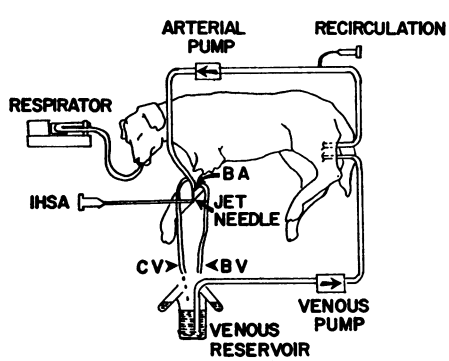

FIGURE 1 Isolated, pump-perfused forelimb. Method for testing the mixing characteristics of the jet needle and comparing indicator dilution calculated blood flow with actual venous outflow in the isolated, pump-perfused dog forelimb.

Experiments in man

The degree of mixing produced by the jet needle was further tested in 39 men. In addition, resting limb blood flow and vascular responses to intrabrachial arterial infusions of magnesium sulfate solution were measured by indicator dilution. All subjects participating in this study were fully informed by the authors of the purposes, procedure, and hazards of the experiment, and written consent was obtained. All subjects participating were male patients at a Veterans Administration Hospital. These patients had normotension or essential hypertension well documented by thorough hospital study, including hospital diastolic blood pressures, averaging above 90 mm Hg during hospital days 4–6, normal rapid-sequence intravenous pyelogram, normal 24-hr urine vinil-mandelic acid (VMA) excretion and normal serum sodium and potassium concentrations (Table I). Severity index in the hypertensives was calculated according to the criteria of the Veterans Administration Cooperative Study (8). All antihypertensive or vasoactive drugs and diets were discontinued at least 4 wk before the response study. No subjects had clinically discernible cardiac or renal insufficiency, acute illness, or other peripheral vascular diseases except mild degrees of asymptomatic arteriosclerosis. An attempt was made to exclude subjects having anomalous bifurcation of the brachial artery by palpating the antecubital fossa.

These male volunteers in the resting, postabsorptive state were studied in an air-conditioned laboratory, with ambient temperature ranging from 24 to 27°C. Before study, the volume of the upper limb distal to the level of the intercondylar line at the elbow was measured by water displacement. With the subject comfortable in the supine position and his arms supported at a 45° angle from the long axis of the body, 20-gauge hypodermic needles with attached plastic tubing were inserted in an upstream direction into the basilic and cephalic veins of one upper extremity (designated "ipsilateral extremity") distal to the elbow. In addition, a 23 gauge needle was inserted upstream into a dorsal metacarpal vein of the ipsilateral extremity in some subjects. In five subjects a fine Teflon catheter was also introduced upstream into a deep antecubital vein. Under local Xylocaine anesthesia the ipsilateral and contralateral brachial arteries and in some cases the ipsilateral radial artery were also cannulated in an upstream direction with 20-gauge Riley arterial needles. In 31 subjects the jet needle was inserted into the ipsilateral brachial artery through the Riley needle and locked into place. The tip of the jet needle usually protruded up to but not proximal to the intercondylar line at the elbow. In an attempt to center the jet in the arterial lumen, the position of the jet needle was manipulated to provide an

TABLE I

Subject List

Subject	Race	Age	Body weight	Limb volume	Mean arterial pressure*	Hct.	Diagnosis‡
		yr	*lb.*	*ml*	*mm Hg*	%	
Normotensines							
J. L. G.	Negro	38	124	1400	88	42	Postviral gastroenteritis
W. H.	Caucasian	48	171	1850	82	42	Duodenal ulcer
C. G. R.	Caucasian	44	141	1425	104	47	Cervical spondylosis
H. W. M.	Caucasian	50	191	1900	98	44	Depression
L. L. D.	Caucasian	45	143	1300	109	37	Chronic pancreatitis
L. T. H.	Caucasian	47	162	1350	83	42	Diabetes mellitus
L. R.	Caucasian	49	155	1700	77	45	Diabetes mellitus
H. C. B.	Caucasian	44	177	1750	93	47	Duodenal ulcer
R. J. N.	Caucasian	35	142	1350	100	49	Chronic pancreatitis
C. L. P.	Caucasian	39	156	1475	94	49	Chronic alcoholism; diabetes mellitus
J. J. C.	Caucasian	53	140	1100	90	46	Laennec's cirrhosis
C. L. C.	Caucasian	44	144	1400	100	39	Laennec's cirrhosis
A. T.	Negro	49	175	1625	90	43	Alcoholic gastritis
W. D.	Caucasian	49	150	1500	100	44	Silicosis
F. L. Z.	Caucasian	61	138	1500	102	42	Lymphoma
R. H. T.	Caucasian	47	171	1300	100	44	Diabetes mellitus
Mean§		45	156	1510	93	44	
Hypertensives							
C. P. J.	Negro	71	172	1600	105	42	Essential hypertension, mild
A. D. R.	Caucasian	49	187	1575	140	45	Essential hypertension, moderate; diabetes mellitus
D. C. O.	Caucasian	46	190	1600	126	46	Essential hypertension, moderate
W. F. C.	Negro	45	148	1500	108	47	Essential hypertension, mild
K. C. V.	Caucasian	54	162	1425	136	44	Essential hypertension, mild
W. E. D.	Negro	43	154	1550	130	45	Essential hypertension, moderate
R. E. B.	Negro	60	128	1300	132	49	Essential hypertension, moderate
J. H.	Caucasian	63	204	1850	140	44	Essential hypertension, moderate
R. L. S.	Negro	44	160	1875	146	47	Essential hypertension, moderate
A. L.	Negro	42	194	1650	147	53	Essential hypertension, moderate
C. D.	Caucasian	46	223	2150	110	50	Essential hypertension, mild
R. S.	Negro	52	228	2250	110	44	Essential hypertension, moderate
J. J. F.	Caucasian	46	136	1400	138	46	Essential hypertension, moderate

TABLE I (*Continued*)

Subject	Race	Age	Body weight	Limb volume	Mean arterial pressure*	Hct.	Diagnosis‡
		yr	*lb.*	*ml*	*mm Hg*	%	
G. E.	Caucasian	51	149	1325	145	41	Essential hypertension, mild
I. S.	Negro	40	150	1550	160	46	Essential hypertension, moderate
J. D. H.	Negro	42	160	1700	135	47	Essential hypertension, mild
F. G. D.	Caucasian	51	217	1850	130	50	Essential hypertension, moderate
R. T.	Caucasian	23	213	1750	115	50	Essential hypertension, moderate
W. W. Z.	Caucasian	47	181	1450	130	50	Essential hypertension, mild
I. L.	Caucasian	48	146	1300	130	49	Essential hypertension, moderate; pulmonary emphysema
W. M. T.	Caucasian	72	150	1250	107	44	Essential hypertension, moderate
O. H. P.	Negro	57	157	1575	120	37	Essential hypertension, mild; diabetes mellitus
J. E. C.	Caucasian	36	178	1600	130	47	Essential hypertension, mild
Mean§		51	176	1704	128	46	

* Mean arterial pressure measured during the experimental procedure by brachial arterial puncture.
‡ Severity of hypertensive disease calculated according to methods of Veterans Administration Cooperative Study (8).
§ Means include only those subjects participating in the response study presented in Table V.

infusion pressure no more than 200 mm Hg above pressure during jet infusion into air. In eight other subjects, intrabrachial arterial infusion was made at 1 ml/min directly through the 20 gauge Riley needle. This latter infusion technique is similar to that used by other investigators.

All infusions in a given subject contained the same concentration of IHSA; total isotope dosage per subject was less than 50 μc. For study of vascular responses to magnesium sulfate, three intrabrachial arterial infusions of equal volume were made: first an isotonic sodium chloride control solution, then the solution of magnesium sulfate (Eli Lilly & Co., Indianapolis, Ind., magnesium sulfate, NF, 10%), then, after a 30 min pause, a second isotonic sodium chloride control solution. The latter infusion was made in 23 of the 26 patients. The 10% magnesium sulfate was diluted with isotonic sodium chloride solution to a concentration of 0.25% (the mean osmolarity of the diluted magnesium sulfate was 289 mOsm/liter). At an infusion rate of 8 ml/min, 0.162 mEq/min of Mg^{++} was infused. Duration of each infusion was 15 min. During infusions, pressures in the ipsilateral cephalic, basilic, and dorsal metacarpal veins, and contralateral brachial artery were recorded in turn, usually at 3, 8, and 13 min. Recordings were made with Statham P23Gb pressure transducers (Statham Industries, Hatorey, P. R.) and with a Sanborn oscillographic recording machine. Ipsilateral cephalic and basilic venous, radial arterial in some cases, and contralateral brachial arterial blood was sampled simultaneously at 5, 10, and 15 min during each infusion. During blood sampling, the cannulae and catheters were first flushed by drawing and discarding 1–2 ml of blood, a volume at least three times the volume of the plastic tubing. Actual drawing of samples, each 2 ml in volume, immediately followed, and these samples were placed in glass test tubes containing dried sodium oxylate. The tubes containing samples were rotated for at least 3 min, and then 1-ml aliquots were pipetted into plastic tubes for radioisotope counting on a Tracerlab crystal scintillation counter.

Calculation of blood flow and vascular resistance

The calculated equation for limb blood flow was an adaptation of that suggested by Andres et al. (1):

$$\text{limb (forearm plus hand) blood flow} = \frac{\text{cpm IHSA infused per min into brachial artery}}{\text{mean cpm/ml venous blood} - \text{cpm/ml contralateral brachial arterial blood}}.$$

The mean indicator concentration of the paired venous samples was used in calculation of blood flow. Mean transit time of indicator across the limb vascular bed was not measured, and therefore the concentration of recirculating indicator was not adjusted for its time intercept. Thus an error was introduced into the calculations. This error was probably of small magnitude, as will be discussed below. In

man, calculated forearm and hand blood flow was expressed as ml/100 cc of forearm and hand volume per minute.

Forearm and hand vascular resistances were calculated as follows: total limb (forearm plus hand) vascular resistance $= \frac{\bar{P}_{BA} - \bar{P}_{LV}}{F}$; limb venous resistance $= \frac{\bar{P}_{SV} - \bar{P}_{LV}}{F}$, where $\bar{P}_{BA}$, $\bar{P}_{LV}$, $\bar{P}_{SV}$, and F represent mean contralateral brachial arterial pressure, mean cephalic or basilic venous pressure, mean dorsal metacarpal venous pressure, and total forearm and hand blood flow per 100 cc of forearm and hand volume per minute, respectively. Resistances were expressed as millimeters of mercury per milliliter of blood flow per 100 cc of forearm and hand volume per minute.

In some subjects contralateral arterial and ipsilateral cephalic venous serum [Na^+], [K^+], [Ca^{++}], [Mg^{++}], and osmolarity, sampled at the 10th min of the infusions, were measured on a Beckman Flame Photometer (model 105), an Advanced Osmometer (model 67-31LAS), and a Perkin-Elmer Atomic Absorption Spectrometer (model 290). Contralateral arterial and ispilateral venous blood hematocrit was also measured.

The Student's t test, simple linear correlation and regression, and the nonparametric median test (9) were used for statistical analyses.

RESULTS

Jet injector needle

Table II presents mean relative differences (mrd) obtained with the various needles, infusion rates, and blood flow rates in tubing simulating the brachial artery (nine experiments). Mean relative difference was significantly reduced ($P < 0.05$) by the jet needle at 8 ml/min as compared with the standard needle at 3 ml/min at all blood flow rates. mrd was also significantly reduced by the jet needle as compared with the standard needle at 8 ml/min at all blood flow rates except 50 ml/min. There was no apparent change in results when the outflow proportions were altered from 50:50 to 60:40. The data in Table II suggest that increasing the volume rate of infusion from 3 to 8 ml/min with the standard needle improved mixing at blood flow rates of 100 ml/min, but the change was statistically significant only at a blood flow rate of 25 ml/min.

TABLE II

Mean Relative Differences between Indicator Concentrations in Branches of Tubing Simulating the Brachial Artery of Man

Infusion technique	Blood flow rate, ml/min				
	200	150	100	50	25
			%		
Standard 20 gauge needle at 3 ml/min	28.0	32.6	32.7	31.8	26.1
Standard 20 gauge needle at 8 ml/min	37.0	31.4	23.5	18.9	16.9
Jet needle at 8 ml/min	13.5*	7.6*	10.0*	10.8‡	5.2*

* Significantly different at $P < 0.05$ from standard needles at 3 and 8 ml/min.

‡ Significantly different at $P < 0.05$ from standard needles at 3 ml/min only.

TABLE III

Suspected Anomalous Bifurcation of the Brachial Artery

Subject	Mean IHSA concentration during resting flow				mrd BV vs. CV
	Contralateral brachial artery	Radial artery	Cephalic vein (CV)	Basilic vein (BV)	
	cpm/ml				%
W. D.	2,766	*	13,226	4,235	75.4
O. H. P.	1,028	*	1,114	4,728	95.4
F. L. Z.	2,092	*	5,561	9,429	35.8
J. E. C.	3,861	3,823	10,710	15,878	27.4
R. H. T.	2,936	2,944	3,112	7,151	91.9

IHSA, iodinated human serum albumin.

* Radial arterial concentrations not measured.

Fig. 2 (*a*)–(*c*) are high-speed flash photographs of Evans blue dye being infused at 8 or 3 ml/min through either the jet needle or a standard 20 gauge hypodermic needle against the flow of water in glass tubing at a water flow rate of 200 ml/min. Mixing of indicator with water appears to be improved by the jet needle. This improvement was also apparent at the other water flow rates used. It may also be seen that most mixing occurred as a result of "rebounding" of the jet streams from the walls of the tube into the main stream.

Experiments in dogs

Hemolysis. Fig. 3 is representative of the pump pressure tracings obtained in the constantly perfused forelimbs of seven dogs during intrabrachial arterial infusions. The top panel is the pump pressure tracing obtained during intrabrachial arterial infusion of isotonic sodium chloride solution at 8 ml/min through a standard 20 gauge hypodermic needle. The center panel is the pressure tracing obtained during an identical infusion through the jet needle. It may be seen that both infusions slightly but equally decreased perfusion pressure and, therefore, limb vascular resistance. Calculated mean decrease in limb vascular resistance was 4.3%.

The limb vascular bed was responsive to products of hemolysis, as indicated in the bottom panel by vasodilation after injection of 2 ml of whole blood through a 27 gauge hypodermic needle into the arterial pump tubing at a velocity which hemolyzed the injected blood. This latter vasodilation was greater and more prolonged than that occurring after an identical injection of isotonic sodium chloride solution. Similar results were found in the other six dogs, a finding suggesting that the jet needle did not create significant hemolysis.

Indicator dilution measurement of blood flow in the isolated, pump-perfused dog forelimb. In five dogs the rd's during intrabrachial arterial jet infusion of indicator solution were (mean ±SD) 3.6 ±2.2%. Fig 4 represents a comparison of actual and calculated flow in the five dogs. Calculated flows were equally accurate at both low and high actual flows; thus, it is unlikely that failure to correct recirculation concentration for mean transit time introduced an error of significant magnitude. In these five dogs calculated blood flows averaged 0.8% above the actual venous outflows. It will be seen that 77% of these observations lay within ±5% of the actual flow, and all observations lay with ±15% of the actual flow. The correlation coefficient was 0.992.

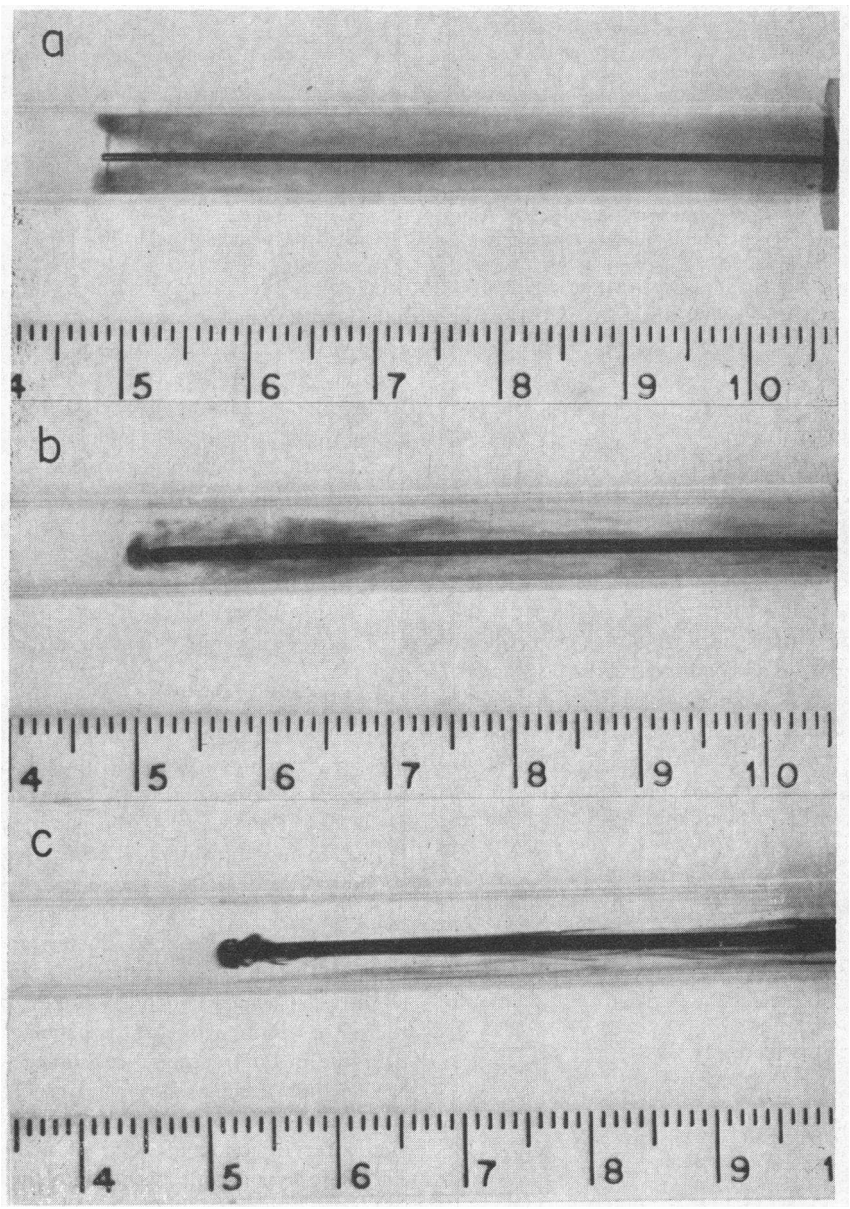

FIGURE 2 Mixing characteristics. (*a*) Infusion of Evans blue dye at 8 ml/min through a jet needle into water flowing from left to right through glass tubing at 200 ml/min. (*b*) Infusion of Evans blue dye at 8 ml/min through a standard 20 gauge needle into water flowing from left to right through glass tubing at 200 ml/min. (*c*) Infusion of Evans blue dye at 3 ml/min through a standard 20 gauge needle into water flowing from left to right through glass tubing at 200 ml/min.

Experiments in man

Data from five subjects were excluded from some of the tables and calculations presented in this section, because there was good evidence of anomalous bifurcation of the brachial artery. In these cases there was a considerable difference between the concentrations of indicator in the paired venous samples. Table III presents data for the five subjects. Findings in subjects O.H.P., F.L.Z., J.E.C., and R.H.T. suggest that indicator may have been infused into the ulnar artery rather than into the brachial artery. In subject R.H.T., isotope concentrations in radial artery and cephalic vein were almost identical with isotope concentrations in contralateral brachial artery, representing recirculating indicator; thus, none of the locally infused isotope passed into the radial artery and cephalic vein. The origin of this subject's radial artery was upstream to the point of indicator infusion, and the radial artery was the sole source of blood to the cephalic vein. The case of J.E.C. is particularly interesting, for here no indicator was infused into the radial arterial blood, and yet mrd was not exceptionally high, a finding indicating that in some subjects a large amount of mixing occurs in the capillary-venous bed of the limb. Findings in subject W.D., in whom most indicator passed into the cephalic vein, suggest that in this case indicator was infused into the radial artery rather than the brachial artery, and that an anomalous ulnar artery supplied the basilic vein.

In 34 other subjects technically satisfactory measurements were made of mixing (mrd) and resting blood flow, and data are presented in Tables IV and V. Comparison of these tables indicates that the mrd was significantly decreased ($P < 0.05$) by the jet injection system, as compared with the standard needle technique, a finding suggesting that the jet injection system improves mixing in vivo as well as in vitro. Note especially subject A.L. in whom both needles were used and in whom mrd decreased from 21.6 to 2.1%. Com-

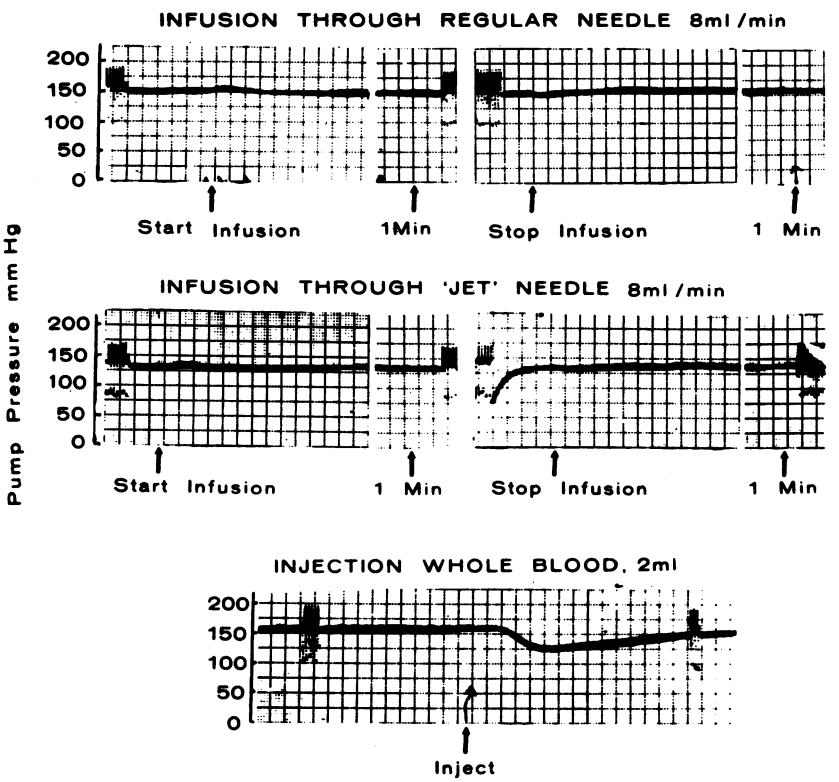

FIGURE 3 Hemolysis. Representative pump pressure tracings during infusion of isotonic saline through standard 20-gauge and jet needles into the constantly perfused dog forelimb. Upper panel: pump pressure tracing obtained during intrabrachial arterial infusion through standard needle. Times of starting infusion, 1 min after starting, stopping infusion, and 1 min after stopping are identified on abscissa. Center panel: pump pressure tracing obtained during identical intrabrachial arterial infusion through jet needle. Lower panel: pump pressure tracing during forceful intrabrachial arterial injection of 2 ml of whole blood through a 27 gauge hypodermic needle.

parison of Tables IV and V also indicates that in all patients calculated mean resting upper extremity blood flows using the two injection systems were not significantly different. This similarity of flows using standard or jet needle further suggests that the jet injections did not produce significant hemolysis and vasodilation. Adjusting flows in these tables by subtracting the infusion rate does not change the conclusions about similarity of blood flows and jet improvement of mixing (mrd).

Infusion directly into the radial artery in four subjects (Table VI) always increased mrd. In addition, radial arterial infusions always increased the concentration of indicator in the cephalic vein and decreased the concentration in the basilic vein. This is further evidence that the source of cephalic venous blood is primarily the radial artery, that the source of basilic venous blood is primarily the ulnar artery, and that the difference in concentration of indicator in the cephalic and basilic veins may be an indication of mixing with arterial blood upstream to the bifurcation. Additional evidence that the choice of veins in the present study provided a sensitive measure of mixing is suggested by data from the five men in whom deep venous blood from an antecubital vein was also sampled. In all five cases the concentration of indicator in the deep vein lay between the concentration values in the superficial veins. Thus, it is probable that the range of concentration values established by the paired samples from the cephalic and basilic veins would include concentration values in most or all other forearm veins.

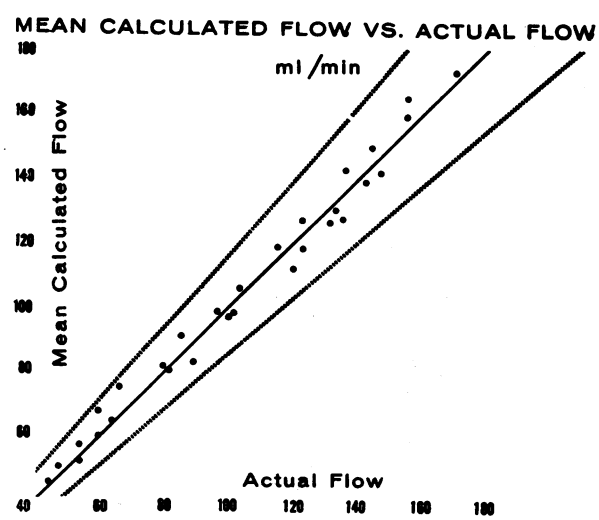

FIGURE 4 Indicator dilution calculated blood flow vs. actual flow (venous outflow) in five isolated, pump-perfused dog forelimbs. Solid regression line represents the line of identity. Broken regression line represents ±15% deviation from the identity line.

TABLE IV

*Mean Relative Difference between Indicator Concentrations in Basilic and Cephalic Veins and Calculated Resting Blood Flow Using Standard Needle**

Subject	Blood flow	mrd
	ml/100 cc/min	%
I. S.	5.3	6.5
J. D. H.	3.3	28.9
F. G. D.	3.0	7.6
R. T.	6.0	14.3
W. W. Z.	4.5	25.2
I. L.	6.6	17.6
W. M. T.	3.9	3.4
A. L.-1	7.2	21.6
Mean ±SD	5.0 ±1.6	15.6 ±9.3

* Subjects with proved or highly suspected anomalous bifurcation of brachial artery excluded.

Table V presents "resting" blood flow and vascular resistance values obtained in the normotensive and essential hypertensive patients during infusion of isotonic sodium chloride solution. Mean ages and forearm and hand volumes were slightly higher in the hypertensive group (Table I). "Resting" blood flows in the two groups were not significantly different ($P > 0.5$). In contrast, even though there was considerable overlap with normotensives, mean vascular resistance in the hypertensives was significantly elevated ($P < 0.05$).

Ipsilateral limb venous serum magnesium concentration, measured in nine subjects, had increased by a mean of 3.39 mEq/liter (range 1.20–5.90 mEq/liter) at the 10th min of the intrabrachial arterial magnesium sulfate infusion. Table V presents vascular responses elicited by the intrabrachial arterial magnesium sulfate infusions. No significant changes occurred in contralateral brachial arterial and ipsilateral cephalic or basilic venous pressures during the infusions, and the mean arterial pressures are presented in Table I. Increase in forearm and hand blood flow and decrease in forearm and hand total vascular resistance occurred in all 26 subjects during the infusion of magnesium sulfate. These changes had occurred by the 5th min of the infusions, and increased slightly further by the 10th min of the infusions. As reported by Baltzan, Andres, Cader, and Zierler (2), a fairly steady state of response had been achieved by the 10th min of the vasoactive infusion, and therefore Table V presents the means of the 10- and 15-min measurements. Mean vascular resistance changes elicited were — 12.14 mm Hg/ml per 100cc per min and — 5.46 mm Hg/ml per 100cc per min in the hypertensives and normotensives, respectively. These changes in blood flow and resistance returned toward or to "rest-

TABLE V

*Limb Vascular Responses to a 15 min Intrabrachial Arterial Jet Infusion of $MgSO_4$**

Subject	Isotonic NaCl, 8 ml/min				$MgSO_4$ 0.25% in isotonic NaCl, 8 ml/min				
	Mean calculated blood flow		mrd	Mean total resistance	Mean calculated blood flow		mrd	Mean total resistance	Δ Resistance
	ml/min	*ml/100 cc/min*	%	*mm Hg/ml/ 100 cc/min*	*ml/min*	*ml/100 cc/min*	%	*mm Hg/ml/ 100 cc/min*	
Essential hypertensives									
C. P. J.	66.9	4.18	7.8	22.08	77.5	4.84	6.4	20.02	− 2.06
A. D. R.	133.4	8.47	12.7	15.24	164.0	10.41	3.8	12.36	− 2.88
D. C. O.	119.3	7.46	12.0	15.52	129.4	8.09	0.8	13.68	− 1.84
W. F. C.	38.6	2.57	2.4	37.18	56.8	3.79	1.4	26.46	−10.72
K. C. V.	69.0	4.85	6.9	25.06	106.7	7.49	6.5	16.13	− 8.93
W. E. D.	89.0	5.75	6.6	20.63	119.8	7.73	7.0	14.98	− 5.65
R. E. B.	18.8	1.45	7.4	76.88	30.2	2.32	7.3	50.87	−26.01
J. H.	66.4	3.59	14.5	37.25	93.5	5.05	2.6	25.38	−11.87
R. L. S.	36.6	1.95	2.0	66.93	55.6	2.96	3.6	43.16	−23.77
A.L. −2	49.6	3.01	2.1	44.83	100.2	6.07	4.0	23.09	−21.74
C. D.	123.9	5.76	3.8	17.97	173.5	8.07	6.1	12.23	− 5.74
R. S.	104.4	3.76	4.4	27.58	139.8	6.21	7.0	16.76	−10.82
J. J. F.	44.8	3.20	1.0	41.57	120.5	8.61	8.1	15.73	−25.84
Mean ±SD	73.9 ±37.0	4.31 ±2.09	6.4 ±4.4	34.52 ±19.38	105.2 ±42.5	6.28 ±2.40	5.0 ±2.4	22.37 ±11.99	−12.14 ±9.12
Normotensives									
J. L. G.	80.7	5.76	4.0	14.10	110.6	7.90	2.2	9.62	− 4.48
W. H.	137.0	7.41	1.9	12.30	133.2	7.20	2.0	11.64	− 0.66
C. G. R.	95.8	6.72	5.6	14.62	143.8	10.09	18.2	9.63	− 4.99
H. W. M.	133.2	7.01	13.2	13.22	152.3	8.02	4.6	11.42	− 1.80
L. L. D.	80.1	6.16	1.8	16.60	90.9	6.99	5.6	15.85	− 0.75
L. T. H.	58.6	4.34	10.3	17.13	74.0	5.48	4.4	13.64	− 3.49
L. R.	33.8	1.99	4.3	33.44	41.0	2.42	1.9	28.16	− 5.28
H. C. B.	104.8	5.99	5.0	13.72	138.8	7.93	0.4	10.72	− 3.00
R. J. N.	46.9	3.47	3.5	26.34	65.0	4.82	7.4	17.94	− 8.40
C. L. P.	37.2	2.52	13.8	32.96	64.6	4.38	2.6	20.64	−12.32
J. J. C.	36.0	3.27	15.6	24.60	73.8	6.71	11.3	11.60	−13.00
C. L. C.	56.3	4.02	6.4	23.02	65.8	4.70	9.8	19.02	− 4.00
A. T.	33.6	2.07	3.6	36.31	46.0	2.83	1.8	27.47	− 8.84
Mean ±SD	71.85 ±36.80	4.67 ±1.93	6.8 ±4.7	21.41 ± 8.60	92.3 ±38.8	6.11 ±2.23	5.6 ±5.0	15.95 ± 6.37	− 5.46 ±4.04

Subjects with proved or highly suspected anomalous bifurcation of brachial artery excluded in this study.
* Mean of 10- and 15-min measurements.

ing" levels during the second infusion of isotonic sodium chloride solution in 18 of the 23 patients in whom this last infusion was made. During this second infusion of sodium chloride mean measured limb vascular resistances were 28.75 and 21.92 mm Hg/ml per 100cc per min in hypertensives and normotensives, respectively. No significant change occurred in limb venous segmental resistance during infusion of magnesium sulfate (mean change + 0.02 mm Hg/ml per 100cc per min) measured in nine subjects. Decrease in venous hematocrit was similar during control isotonic sodium chloride (− 6.0%) and magnesium (− 4.7%) infusions, measured in eight subjects, a finding suggesting that decrease in resistance in response to magnesium sulfate is not attributable to change in viscosity. Decreases in venous hematocrit during control isotonic sodium chloride infusions were similar to the expected decrease in hematocrit if there had been a simple addition of the infused solution to the initial blood flow. This latter finding thus supports that of Wahren (4).

Small changes occurred in limb venous serum osmolarity and concentrations of sodium, potassium, and calcium during control isotonic sodium chloride infusions (− 1.8 mOsm/liter, + 3.4 mEq/liter, − 0.6 mEq/liter, and − 0.83 mEq/liter, respectively [n = 9]). Similar small changes occurred during infusions of magnesium sulfate (− 1.7 mOsm/liter, + 1.6 mEq/liter, − 0.6 mEq/liter, and − 0.63 mEq/liter, respectively [n = 9]), a finding suggesting that the vascular effect of magnesium sulfate is not mediated via changes in serum osmolarity or concentrations of other vasoactive electrolytes.

Decrease in total forearm and hand vascular resistance was significantly greater ($P < 0.05$) in hypertensives than in normotensives by either parametric or nonparametric statistical tests. However, in both hypertensives ($r = 0.891$; $P < 0.01$) and normotensives ($r = 0.714$; $P <$

TABLE VI

Mixing During Radial Arterial Infusion of Indicator

Subject	Mean relative difference in per cent between indicator concentrations in basilic vein and cephalic vein	
	Infusion into radial artery	Infusion into brachial artery
J. L. G.	28.0	3.6
A. L.-2	18.0	1.7
G. E.	53.1	22.4
C. G. R.	100.0	5.5
Mean	49.8	8.3

0.01) there was a significant correlation between level of resting limb vascular resistance and magnitude of resistance change evoked by magnesium sulfate. If magnitude of vascular response to magnesium sulfate is plotted against the level of initial vascular resistance (Fig. 5), the regression coefficient of the response points in hypertensives ($b = 0.419$) is not significantly different from the regression coefficient of normotensive response points ($b = 0.335$; $P > 0.5$). Mean responses in hypertensives adjusted for this regression on initial resistance were also not significantly different from normotensive mean responses similarly adjusted ($P > 0.2$).

DISCUSSION

The results of the present study suggest that total forearm and hand blood flow in man may be accurately calculated by the constant infusion indicator dilution technique described, provided that anomalous high bifurcation of the brachial artery is not present. In addition, the results of the present study suggest that the jet injection system used improves mixing of indicator and other substances with brachial arterial blood. The study also provides evidence suggesting that there may be a large degree of physiological separation of the vascular bed supplied by the radial artery from that supplied by the ulnar artery. Finally, the study confirms previous reports that limb vascular resistance is elevated in hypertension and defines the response of the limb vascular bed to local alteration of blood magnesium concentration in normotensives and essential hypertensives. Evidence is presented for a correlation between initial limb vascular resistance and magnitude of response to magnesium. The relationship of this correlation to interpretation of vascular response data is discussed.

The present study confirms previous reports supporting the validity of constant infusion, indicator dilution

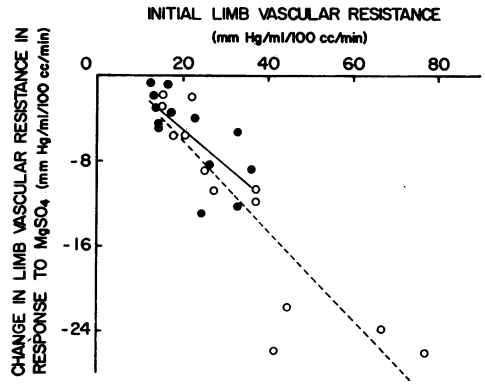

FIGURE 5 Initial vascular resistance vs. magnitude of vascular response to $MgSO_4$ infusion. Solid circles represent responses in hypertensives. Regression equation for normotensives: $y = -0.335x + 1.71$. Correlation coefficient for normotensives $= 0.714$ ($P < 0.01$). Regression equation for hypertensives: $y = -0.419x + 2.32$. Correlation coefficient for hypertensives $= 0.891$ ($P < 0.01$).

measurement of limb blood flow (1, 4, 10, 11). Results from the isolated, pump-perfused dog forelimb indicate that blood flow may be measured with considerable accuracy by means of this technique.

Results of the present study further suggest that, without causing hemolysis, the jet injection system improves mixing of substances with arterial blood over that produced by standard infusion systems; use of the jet injection system decreased the difference in concentration of indicator in downstream vessels (rd) both in vitro, in tubing simulating the brachial artery, and in vivo, in the limbs of men. In the limbs of men considerable mixing may occur in the capillary-venous bed; however, it seems unlikely that the over-all degree of capillary-venous mixing was different in the groups of subjects in whom the jet and standard injection systems were compared (Tables IV and V). Therefore, mixing with arterial blood was probably improved.

Because the jet injection system does not achieve levels of infusate kinetic energy necessary to produce turbulence (1, 11), the improved mixing must be attributed to another mechanism. That this mechanism is related in part to volume rate of infusion is supported by the in vitro data and also by the studies of Wahren (4). From the photographs of mixing in glass tubing, it appears that indicator is also mixed by being spread across the laminae of flow by the jet effect. Rather than converting laminar flow into turbulent flow, the injection system appears to act by dispersing indicator into the various laminae of flow or by accelerating diffusion of indicator. It may be possible to further improve the degree of mixing of the jet injector by increasing the number and decreasing the diameter of orifices or by changing the shape or angle of the orifices.

The data also indicate a large degree of physiological separation of the vascular bed supplied by the radial artery from that supplied by the ulnar artery. Infusion into the radial artery resulted in appearance of increased indicator concentrations in the cephalic vein and decreased concentrations in the basilic vein. Thus, adequate mixing of vasoactive substances with arterial blood upstream to the bifurcation of the brachial artery is necessary for the reliability of vascular response studies. Any system which improves arterial mixing should improve this reliability. It is also essential to detect satisfactory mixing in such response studies, especially in order to exclude anomalous high bifurcation of the brachial artery (2). The indicator dilution system described in the present paper offers both advantages.

The blood flows presented in Tables IV and V are unadjusted for intrabrachial arterial infusion rate. The infusion rate of 8 ml/min probably reduces limb vascular resistance slightly (about −4% in the pump-perfused dog forelimb) and increases limb blood flow. These changes are produced by reduction in blood viscosity and perhaps also by increases in intrabrachial arterial pressure and decreases in concentrations of vasoconstrictor chemicals. However, the absolute increment in flow is uncertain, although data from the present study tend to support the findings of Wahren (4), suggesting a simple addition of the infused solution to the initial blood flow.

Use of this constant infusion, indicator dilution technique to measure resting vascular resistance in man confirms plethysmographic observations that there is considerable overlap in limb vascular resistances between normotensives and hypertensives, but that mean resistance in the hypertensive group is significantly greater than that in the normotensive group (12, 13). Thus, the limb vascular bed appears to participate in the increase in total peripheral vascular resistance in hypertension, suggesting that the limb vascular bed should participate in any abnormalities in vascular responses existing in hypertension.

In man limb vascular responses to local infusions of magnesium sulfate are similar to those in the pump-perfused forelimb of the dog (5). Previous data suggest that the sulfate ion does not produce significant vasoactivity (5). Thus in man the effect of local increases in serum magnesium ion concentration is a decrease in vascular resistance, probably due to active vasodilation. It follows that the magnesium ion may be considered an endogenous vasodilator similar to bradykinin, histamine, and acetylcholine.

Limb vascular response to the magnesium ion, unadjusted for the different levels of initial vascular resistance in the two groups, is significantly greater in essential hypertensives than in normotensives.[1] This exaggerated response to exogenous magnesium ion suggests hyperresponsiveness to endogenous magnesium (and possibly to other endogenous vasodilators) in hypertension. These exaggerated vasodilator effects should tend to oppose the exaggerated responses to endogenous vasoconstrictors reported to exist in hypertensives (6). Thus interpreted, the present data might suggest an abnormality in vascular wall magnesium metabolism in hypertension; it has been similarly suggested that hyperresponsiveness to catecholamines in hypertension may be attributed to an underlying defect in vascular wall sodium metabolism (6).

On the other hand, in man a significant positive linear correlation between level of initial limb vascular resistance and magnitude of limb vascular response to vasoconstrictor agents has been reported (13). In the present study the data from both normotensive and hypertensive groups indicate that there is a similar significant positive linear correlation between initial resistance and magnitude of dilator response to the magnesium ion. The correlation is similar to that found in the response of the dog forelimb, pump perfused at constant flow, to local magnesium infusions (14). This similarity suggests that the correlation found in the limb of man is not solely an effect of dosage (e.g. within the normotensive group a low resistance limb tends to have a high blood flow, and thus a constantly infused vasoactive agent would be more diluted than in the case of a high resistance limb with a low blood flow). If the correlation between initial resistance and magnitude of response is considered in interpretation, the present data fail to provide evidence of an abnormality in the hypertensives' vascular response to the magnesium ion. This interpretation assumes that the normal linear relationship between initial resistance and magnitude of response does not become curvilinear at high levels of resistance. There is data from the pump-perfused forelimb of the normotensive dog (with limb resistance artificially elevated by local nerve stimulation, local angiotensin II infusion, or hemorrhage) suggesting that the assumption of linearity at high resistance levels is correct for the case of a vasodilator agent (15).

From the above discussion it is clear that conclusions about vascular wall magnesium metabolism in hypertension drawn from these data depend on the method of interpretation. If the relationship between initial resistance and magnitude of response is considered, the conclusions are opposite those if the relationship is ignored. Evidence has been presented indicating that ini-

[1] It is unlikely that this response difference could be explained by a difference in Mg^{++} binding, because plasma proteins and pH are reported similar in normotensives and essential hypertensives.

tial resistance is a significant source of variation in the present data. Therefore we favor considering initial resistance in interpretation and thus conclude that the limb vascular response to the magnesium ion appears to be similar in essential hypertensives and normotensives. Thus interpreted the present data would fail to suggest an abnormality in vascular wall magnesium metabolism in hypertension. However, these results do not preclude the possibility that there may be an abnormal vascular response to the magnesium ion at other dosage levels or in other forms of hypertension, steroid or renal for example.

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