Visualization of Tumor Vascularity in a Rabbit VX2 Carcinoma by Doppler Flow Mapping

Jonathan M. Rubin, MD, PhD*, Paul L. Carson, PhD*, Robert A. Zlotecki, RPh†, William D. Ensminger, MD, PhD†

Using an ultrasonic dynamic flow imager that displays both soft tissues and color-coded flow in the same two-dimensional slice, we were able to display neovascularity in a rabbit VX2 carcinoma. Intravenous infusion of epinephrine altered the flow dynamics in two arteries, one within the tumor and one at the periphery. Further, we were also able to visualize areas of multidirectional flow presumably due to complex arterial patterns and arteriovenous shunts. It is concluded that the color-coded Doppler instrument may overcome some of the methodological problems associated with tumor diagnosis via flow characteristics in the human breast. The literature indicates that the vascular response to the vasoactive drugs or thermal stress may increase differentiation of malignant breast lesions. This experiment suggests that Doppler images and measurements may be made efficiently with color-coded Doppler images, particularly with the addition of more quantitative features to the imager. KEY WORDS: ultrasonography; tumors; tumor imaging; breast cancer. *(J Ultrasound Med 6:113, 1987)*

Cancer diagnosis by Doppler ultrasound has been studied for several years.[1-5] In breast cancer, in particular, when 10 MHz continuous wave (CW) Doppler units were employed, approximately 90% of localizable tumors exhibited abnormal flow properties.[1-4] Among these were very high peak velocities, increased mean velocities, and, perhaps most characteristic of cancer, high spectral power at a wide range of frequencies, including forward and reverse flow.

Burns et al studied a vascular tumor, VX2 carcinoma, implanted on rabbits' ears to discern the source of the abnormal flow patterns.[6] They saw spectra and peak velocities indistinguishable from those in human breast carcinoma. High blood velocities and high diastolic flow were attributed to local pressure drops, usually resulting from arteriovenous shunting. The intersection of numerous small vessels with multiple orientations by the continuous wave ultrasound beam was judged to be responsible for the observation of large signals over a wide range of positive and negative Doppler shift frequencies. It has been shown by magnification angiography and mathematical modeling that much of this signal results from vessels of several hundred microns in diameter.[7] (In our own very limited experience with continuous wave 10 MHz Doppler on VX2 carcinoma in the rabbit thigh, it has been unclear whether the abnormal signals we detected were due to the multiple small arteries in and around the tumor or to a few major feeder arteries, although the opposite normal thigh produced no such abnormal signals.)

A limitation of Doppler techniques for breast cancer detection has been the difficulty of searching over the

Received December 31, 1986; from the Departments of *Radiology and †Internal Medicine and Pharmacology, University of Michigan Hospitals, Ann Arbor, Michigan. Revised manuscript accepted for publication May 10, 1986.

Address correspondence and reprint requests to Dr. Rubin: Department of Radiology, University of Michigan Hospitals, Ann Arbor, MI 48109-0010.

large volume of amorphous breast tissue for tumor vessel flow and distinguishing it from normal arteries. Wells et al have worked on an array of continuous wave transducers for covering a large area of the breast at one time,[8] but no clearly positive results have been reported as yet. The real-time color flow mapping image, superimposed with a very high resolution pulse echo image, as evaluated here, is potentially a more versatile and effective method of identifying a possible abnormality and evaluating its flow characteristics.

A limitation of imaging pulsed Doppler, in comparison with CW Doppler, is a reduced sensitivity to flow due to a larger band width and a smaller amount of flowing blood in the sample volume with the pulsed Doppler technique. For tumor detection, it might be possible to change to a high sensitivity, low resolution mode where the band width is narrowed and the sample volume extended to around 1 cm in a region of interest defined by the normal image.

MATERIALS AND METHODS

The experiments were performed on a 2.5 kg, female, New Zealand white rabbit that had a 1.0 mg implant of VX2 carcinoma placed into the right thigh 20 days prior to the procedure. At the time of the experiment, several palpable solid tumor nodules had developed, the largest being 3–4 cm in greatest dimension. The animal was lightly sedated with an i.m. injection of 75 mg ketamine (Ketalar, Parke-Davis) and 15 mg xylazine (Rompun, Miles Laboratories) into the left gluteal muscles. The fur covering the largest tumor mass, the right inguinal region, the left inguinal region, and the ventral surface of the lower abdomen was sheared.

Scanning was then performed using a prototype Quantum Medical Systems Ultrasound Angiodynograph 1 (Quantum Medical Systems, Issaquah, WA). The device uses a 7.5 MHz linear phased array probe containing 92 individual ultrasonic transducer elements, and it has the ability to display both soft-tissue gray scale and flow simultaneously in the same image at 18 frames/sec. A fast-Fourier transform is performed on the received signal across the image. The centroid or arithmetic mean of the Doppler frequency shifts within each pixel is color coded. Flow towards the scanhead was fixed as shades of red, flow away as shades of blue. The higher the velocity of flow, the whiter the hue becomes.

We scanned systematically above the tumor mass in the right thigh and right inguinal region, on top of the tumor itself, and in the normal left inguinal region. Vessels were identified in all areas. After identifying a pulsatile vessel at the upper margin of the mass, a continuous i.v. epinephrine (adrenaline chloride, Parke-Davis) infusion at 3 μg/min was administered over a 2 min 6 sec period through an ear vein using a 25-gauge butterfly cannula while continuously monitoring the flow characteristics within the marginal artery. After a 9-min delay, another vessel with high pulsatile flow was identified within the mass itself, and 2 μg boluses of epinephrine were given at time points of 0 min, 1 min 26 sec, 2 min 40 sec, 4 min 20 sec. This vessel was continuously monitored during the injections. The entire procedure was recorded on ½-inch VHS videotape.

The flow data were evaluated with a frame-by-frame analysis of the videotape record. The fraction of the cardiac cycle during which flow could be detected (flow fraction) in the vessel at the margin of the tumor and the vessel within the tumor was determined. A cardiac cycle was defined as the time period between adjacent instances of the appearance of flow in a given vessel. The flow fraction could be determined by single framing through the video record of the experiment. Actually, individual video fields were accessed when "single framing," so the time resolution was 1/60 sec. By counting the number of individual video fields between the onset of flow in one beat to the onset in the next and then counting the number of fields during which flow was observed, the percentage of each beat containing flow could be determined. This proportion was averaged over time intervals of 10–20 sec to minimize the variance and was plotted on a scale which indicates time of epinephrine infusion(s). The apparent pulse rate was also determined from the videotape record. The rate of appearance of flow in the two arteries was calculated using a digital stopwatch to time the videotape record. This value is less than or equal to the actual pulse rate due to the possibility of missed beats secondary to nonvisualization during severe vasoconstriction. The constraints of this preliminary experiment did not allow us to monitor the pulse rate itself, but we can still use this value as a relative, nonlinear measure of the rate of blood flow through an imaged vessel.

The flow fraction is dependent on machine settings, in particular, the system gain, output, and the color scale mapping of the Doppler frequency shifts. The other standard parameters affecting Doppler spectra, such as sample size, vessel size, and angle of incidence, will also affect the flow fraction. Hence, flow fractions are useful only as relative measures, comparing a vessel with itself after some intervention, and to a lesser degree, comparing one vessel with another.

RESULTS

The primary tumor mass was approximately 6.5 cm in thickness (Fig. 1). Scanning was performed over the pri-

mary tumor mass on the outside of the right thigh, at the margin of the tumor, and along the course of the right iliac into the right common femoral artery. The left inguinal region and the lateral margin of the left thigh were also scanned as controls.

Several discrete areas of flow were demonstrated within the tumor. Some of these appeared multidirectional, showing multiple variations of reds and blues (Fig. 1). However, diffuse areas of increased flow throughout the tumor were not identified. We therefore could not with certainty detect tissue perfusion within the tumor mass or the normal limb. Areas of flow were identified where there was no recognizable vascular channel in the stationary gray scale component of the image (Fig. 2). Hence, these small vessels could be demonstrated only by their internal flow characteristics.

Vessels were also identified peripheral to the tumor (Fig. 3). They too were usually identified by observing pulsatile flow within them. The one major exception was the much larger, approximately 2 mm diameter, external iliac artery leading into the common femoral artery (Fig. 4). The areas of apparent multidirectional flow demonstrated within the tumor were not observed either in the areas of the right limb above the tumor mass or in the opposite normal left limb.

In order to evaluate ultrasound's ability to detect blood flow-altering affects of epinephrine, the flow properties within two arteries, one just external to the margin of the mass (Fig. 3) and one within the mass (Fig. 2), were assessed. We determined that the chosen vessels were arteries by assessing their rapid, pulsatile flow dynamics and that their flows were unidirectional. This was easily done by locating a candidate vessel and observing its flow over time. Certainly, the direction of flow toward or away from the lower limb would discriminate normal arteries from veins, but within the tumor, this type of directional reliability did not hold. The vessel within the tumor selected for analysis clearly had flow from outside the tumor inward, suggestive of an artery or tumor vessel but not a draining vein. We were unable to trace the small vessels within the tumor to the periphery of the mass, however.

The flows in these two vessels were monitored before, during, and after i.v. infusion of epinephrine. The flow fractions within the two vessels are shown (Figs. 5 and 6). The associated pulse rates are also plotted (Figs. 7 and 8). There was a clear frame-to-frame difference in the amount of flow through both arteries with and without epinephrine infusion with either the bolus or continuous infusion technique. During both types of infusion, the number of color-coded pixels across the vessel being interrogated changed without any obvious whitening in hue in those pixels still displaying flow. Since there was little, if any, change in hue of those pixels displaying flow during the experiment, any change in the imaged diameter of these vessels is a relative measure of the amount of flow in a vessel. Although only a qualitative measure in this prototype unit, there was a definite decrease in the cross-sectional area of pixels defining the artery at the margin of the tumor during the epinephrine infusion with little or no change in the cross-sectional pixel area of the art within the tumor.

The vessel at the margin showed marked changes in flow dynamics before, during, and after the continuous epinephrine infusion (Fig. 6). Within 5 sec of the initial epinephrine infusion, the quality of flow changed dramatically. The flow within the vessel jumped until it became almost continuous as manifested by the spike in the apparent pulse rate, while the flow fraction increased to 63% with a standard deviation of ± 16%. After 10 sec, both parameters had fallen below their control levels. The fall of the flow fraction (20% ± 7% to 4.9% ± 3.1%) was significant ($P < 0.001$, two-tailed t test) (Fig. 6). Also, the average flow fraction during the epinephrine infusion, 8 ± 6%, was also different from the control ($P < 0.001$). The flow fraction returned to base-

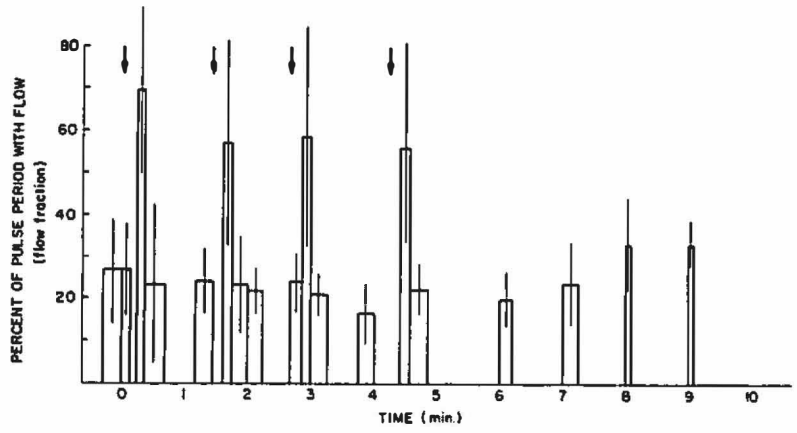


Figure 5 Flow fraction in artery leading into the center of the tumor (Fig. 2). Four equivalent 2 μg boluses of epinephrine were injected at instances marked by arrows. (Error bars represent standard deviations).

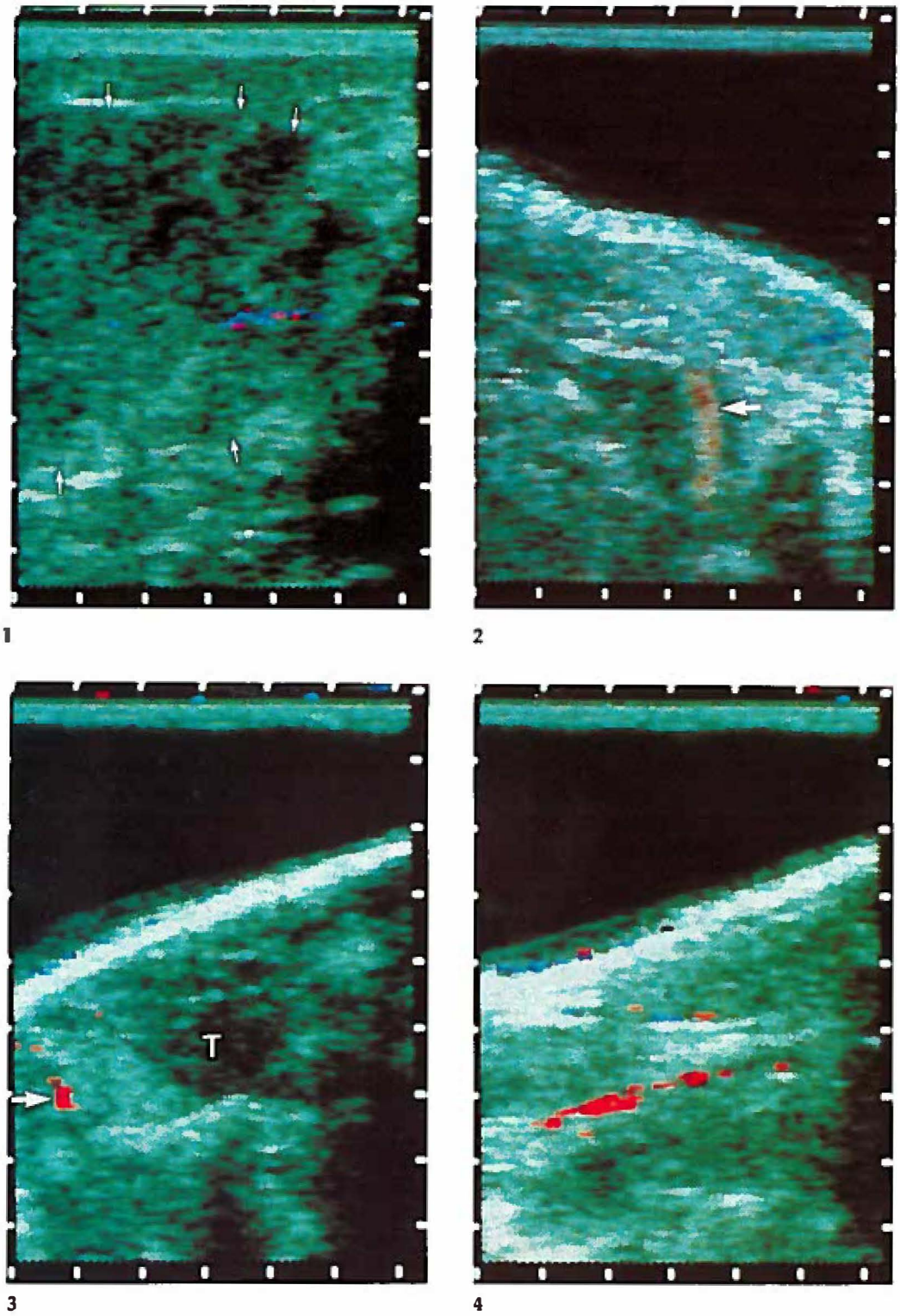
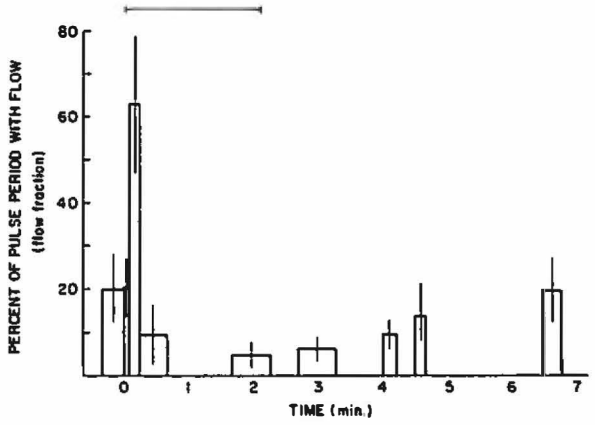


Figure 1 Scan through tumor mass showing a local area with multi-directional flow manifested by an isolated collection of red and blue pixels in the center of the image. The anterior and posterior margins of the tumor are marked with arrows. This collection did not have any intrinsic periodicity and the appearance and disappearance of flow within small areas in the collection did not correlate. These findings suggest that it might represent multiple small vessels or less likely, multiple areas of enhanced perfusion.

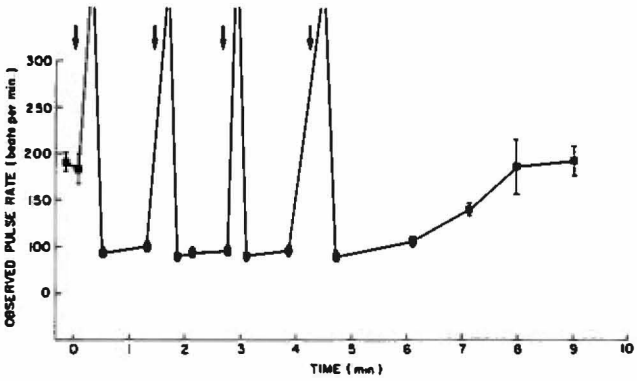
Figure 2 Scan showing a large vessel flowing through the tumor mass *(arrow)*. No vascular channel was visible, and this vessel was only detected by its flow; however, the orientation of the vessel is approximately 90° to the scanhead, making visualization of the vessel walls less likely. Note: A fluid path of tapered length and attachable to the scanners was used in Figs. 2–4.

Figure 3 Longitudinal (cephalad to the left) scan showing a small artery *(arrow)* near the cephalad margin of the relatively anechoic tumor (T).

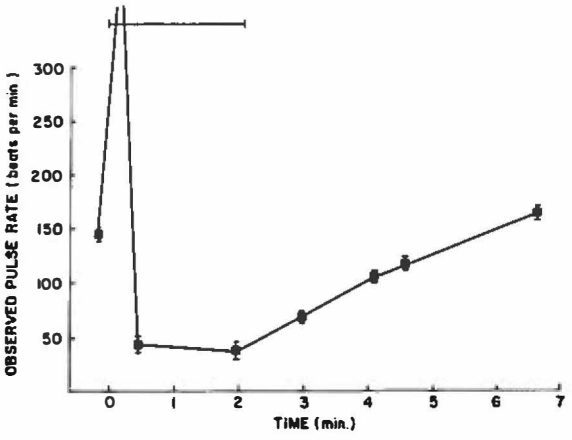
Figure 4 Longitudinal scan through the external iliac artery showing cephalo-caudad flow.



6



7



8

Figure 6 Flow fraction in artery at the upper margin of the tumor that was demonstrated in Fig. 3. The infusion of epinephrine at 3 μg/min was given continuously over the initial 2 min 6 sec as indicated by the bar at the top of the graph.

Figure 7 Measured pulse rate through the artery leading into the center of the tumor. After each bolus, the flow became almost continuous. This phenomenon is represented by the four discontinuous peaks in the graph, which probably do not represent an extended increase in the pulse rate (see Addendum). (Arrows mark the injection times.)

Figure 8 Observed pulse rate through the vessel at the cephalad margin of the tumor. The single episode of near continuous flow is shown in the discontinuous peak in the record. (Bar at top of graph denotes period of epinephrine infusion.)

line within approximately 5 min of cessation of the infusion. There was a marked diminution of the observed pulse rate in the marginal vessel from 145 ± 6 beats/min to 38 ± 7 beats/min during the epinephrine infusion ($P < 0.001$). This also recovered within 5 min of the cessation of the infusion. Although there is a clear rebound effect on the pulse rate, the flow fraction returned to a level not significantly different from control after discontinuing the infusion ($P > 0.5$).

The vessel within the tumor mass responded somewhat differently. After each bolus of epinephrine, the pulse rate and the flow fraction increased similarly to the first vessel. These peaks lasted only a few seconds, and then the pulse rates quickly fell below the baseline level while the flow fractions returned rapidly to just slightly below normal levels. The mean value of the flow fractions between the four postbolus spikes was 22 ± 12%, which was only mildly different from the preinfusion level ($P < 0.025$).

On the average, there was a greater depressive effect of epinephrine on the flow fractions observed in the vessel at the tumor's margin than on the vessel within the tumor (8.0 ± 6% vs. 22 ± 11%, $P < 0.001$). Further, unlike the vessel at the tumor's margin, the quantity of flow as subjectively estimated by the image area demonstrating flow across the vessel within the tumor did not change from control levels. After the cessation of epinephrine infusion, an interesting rebound effect occurred in the artery within the tumor. The pulse rate increased above baseline as in the marginal artery, but the flow fraction clearly recovered to baseline or above.

DISCUSSION AND CONCLUSIONS

Real-time imaging of the flow in arteries as small as ≤ 1 mm opens many possibilities for improved diagnosis. Most exciting, however, is the possibility of noninvasive visualization of induced changes in the response of normal and tumor vessels to small, i.v. doses of a vasoconstrictor which is expected to have different effects on blood flow in normal tissues vs. tumors.[9] Intravenous administration of vasoconstrictors for low-risk diagnostic applications requires considerable additional investigation to establish whether safe doses of vasoactive agents will have useful effects. Certain patients, including those with various cardiac and respiratory disorders, probably would have to be omitted from these otherwise low-risk studies.

An intriguing alternative to drugs is the use of the sympathetic nervous system for differential constriction of normal vasculature. Immersion of the hands and wrists in ice water for 15 sec has been employed in stress thermography and graphic stress testing to reduce flow

in superficial vessels, possibly providing higher skin temperature contrast for veins returning from breast tumors.[10] It has been hypothesized that the sympathetic nervous system constricts deeper normal arteries in the breast as well as superficial ones.[11] While this hypothesis has not been tested by thermography, which is insensitive to all but very superficial vessels, its truth is supported by the experience of at least one group working with Doppler ultrasound.[11] Their observed effects on breast tumor flow from chilling some part of the upper extremities, with less extreme cold than ice water, could be safely and easily confirmed. If the hypothesis is true, confirmation of the neovascular nature of tumor vessels with color-coded Doppler flow mapping would be relatively fast and simple.

The ability to detect neovascularity along with soft-tissue detail should be of significant help both in localizing tumors in sites such as the breast and in characterizing masses once they have been found. Continuous wave Doppler scanners can detect aberrant flow patterns that are highly specific for carcinomas,[1-4] but the tedious searches required to locate the tumors with the associated low sensitivity for detection makes the procedure impractical. However, we suspect that the use of a dynamic flow imager, such as the one we employed, could greatly improve the sensitivity, possibly making this a major adjunct to the present methods for diagnosing breast cancer.

The actions of epinephrine are complex and varied depending on many factors. We were, however, able to detect a marked and substantial increase in the flow fraction immediately after either bolus injection or continuous infusion which could be manifestations of epinephrine's marked positive inotropic effect along with its known effect of increasing blood pressure.[12] The subsequent rapid drop in both flow fraction and pulse rate in both vessels is possibly because these effects are quickly negated by an epinephrine-induced increase in peripheral resistance and a compensatory deceleration of the heart rate due to vagal discharge.[12]

The flow fraction fell below control levels in both arteries, although significantly more in the artery at the margin of the tumor. Approximately 4–5 minutes after cessation of epinephrine, there apparently was a decrease in peripheral resistance with an associated decrease in vagal tone and an increase in pulse rate. The flow fraction returned to normal in both vessels with an added rebound in the vessel within the tumor.

The lesser epinephrine effects on the vessel within the tumor appear to have been an indication and reflection of that vessel's tumor angiogenic origin, although the lesser effects may have been due to the differences in the epinephrine regimes, including the fact that the marginal vessel was studied first. We doubt that the system had become refractory to epinephrine between the first infusion and the series of boluses, since the marked increase in the flow fraction after the initiation of the continuous infusion was not different from the mean of the four flow fraction increases recorded after the bolus infusions, $63 \pm 16\%$ vs. $62 \pm 23\%$ ($P > 0.5$) or from the increase after the first bolus alone, $63 \pm 16\%$ vs. $70 \pm 20\%$ ($P > 0.1$). We therefore suspect that the vessel within the tumor may be the result of tumor angiogenesis or at least supplies a larger fraction of its flow to tumor tissue than does the artery at the tumor margin. The proof of this supposition will require further study using a standard injection method throughout the experiment. However, this experiment definitely demonstrated the ability to detect small arteries in and around tumors as well as flow changes induced in them by vasoactive substances. As mentioned, there was some depression of the flow fraction within the presumed tumor feeder vessel. This could be due to the large doses of epinephrine resulting in increased vascular resistance upstream in the normal vessels and diminished cardiac output.

Very carefully controlled human studies will be required to determine reliable vasoconstriction methods to easily reveal the differences between normal vessels and neovasculature and to determine the specificity of neovascular-type responses for distinguishing malignant processes.

We devised a relative measure, flow fraction, to permit some quantitation of the changes in flow patterns in the prototype machine. Clearly, this measure is dependent on multiple technical factors including system gain, output, color-scale mapping of Doppler frequency shifts (Figs. 2 and 3), sample size, vessel size, and angle of incidence, to name a few. All of these are easily controlled within a given vessel, but may be difficult or impossible to completely standardize between vessels. Hence, the technique has strong, consistent utility only in describing changes within a given vessel. If the differences in two vessels are dramatic or the vessel size, orientation, depth, and initial flow characteristics are very similar, meaningful comparisons might also result. On this latter basis, we think that the weak statements comparing the two vessels studied are justified, since their respective flow fractions (Figs. 5 and 6) indicate the two systems were approximately in equivalent states before the epinephrine injections. Certainly, further experiments are needed to verify these points.

ADDENDUM

Subsequent to the submission of this paper, we have repeated the bolus experiment on another rabbit with a VX2 carcinoma in the right thigh. This time, however, the machine had been upgraded to permit the perform-

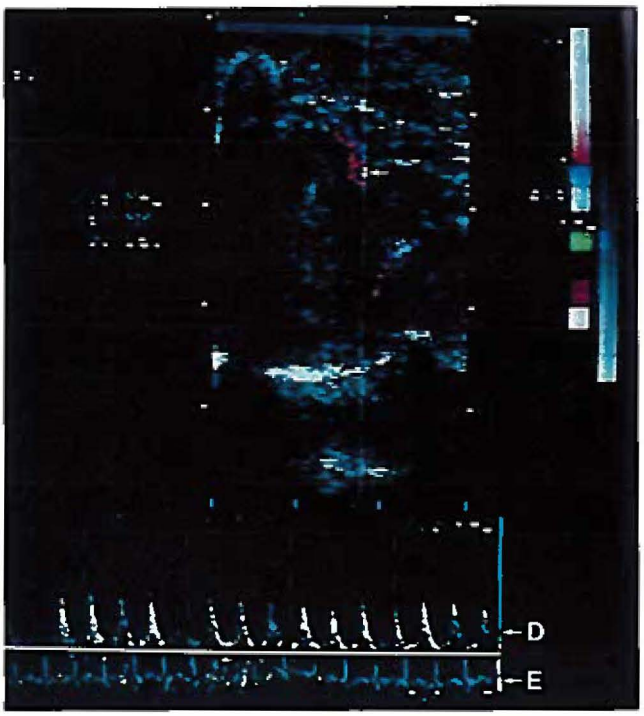


Figure 9 Preepinephrine image of an artery running through a VX2 carcinoma mass in a rabbit's thigh. A Doppler sample volume *(arrow)* is placed within the vessel, which is red. The Doppler spectral tracing (D) and EKG tracing (E) are seen below.

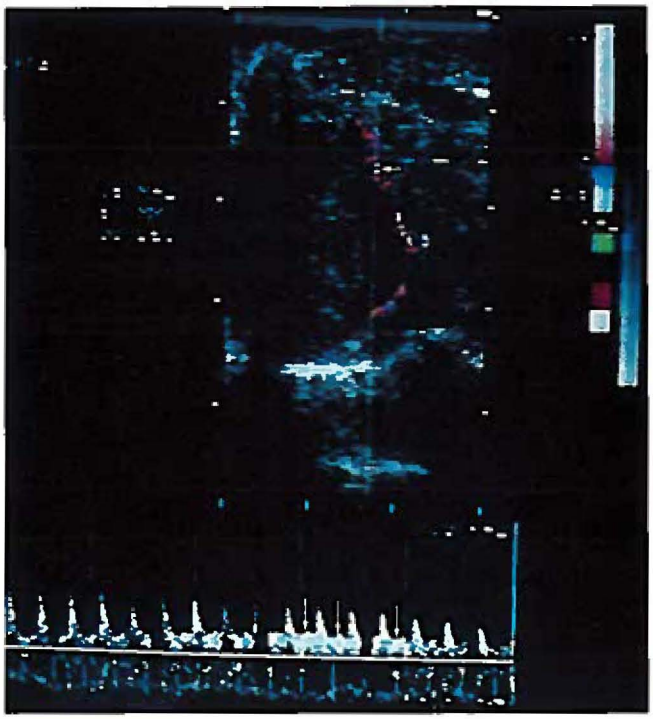


Figure 10 Approximately 10 seconds after 2 μg of epinephrine is injected into an ear vein, there is a marked increase in the diastolic flow in the artery *(large arrow)*, yet the EKG tracing has hardly changed. The sample volume *(small arrow)* is seen within the artery, color coded as red.

ance of spectral analysis at up to two selected locations within the image simultaneously. We identified an artery, based on its flow pattern, running into the VX2 carcinoma (Fig. 9). Approximately 10 seconds after injection of a 2 μg bolus of epinephrine into an ear vein, there was a marked increase in the diastolic flow in the vessel (Fig. 10). This increase corresponded precisely with the marked increase in flow fraction and apparent increase in pulse rate observed in the flow images immediately after epinephrine infusion in the first rabbit. The pulse rate, based on simultaneous EKG monitoring, hardly changed. Therefore, we think that the apparent increase in pulse rate on the flow images was due to diastolic flow rising above the flow display threshold. The observed marked increase in flow fraction was due to an immediate, dramatic increase in blood pressure with no increase in peripheral resistance secondary to the epinephrine infusion. This interpretation is supported by recent measurements on VX2 carcinoma in rabbit liver.[14]

ACKNOWLEDGMENT

This study was supported in part by NIH grant CA33825.

REFERENCES

1. Wells PNT, Halliwell M, Skidmore R, Webb AJ, Woodcock JP: Tumor detection by ultrasonic Doppler flow signals. Ultrasonics 15:231, 1977
2. Burns PN, Halliwell M, Wells PNT: Ultrasonic Doppler studies of the breast. Ultrasound Med Biol 8:127, 1982
3. White DN, Cledgett PR: Breast carcinoma detection by ultrasonic Doppler signals. Ultrasound Med Biol 4:329, 1978
4. Minasian H, Bamber JC: A preliminary assessment of an ultrasonic Doppler method for the study of blood flow in human breast cancer. Ultrasound Med Biol 8:347, 1982
5. Boyd J, Jellins J, Reeve PS, Kossoff G: Doppler examination of the breast. *In*: Jellins J, Kobayashi T (eds): Ultrasonic Examination of the Breast. New York; John Wiley & Sons, 1983, pp 371–378 (see related articles in the same volume)
6. Burns PN, Virjee JM, Gowland M, Rimmer S, Wells PNT: The origin of Doppler shift signals from breast tumors. *In*: Jellins J, Kobayashi T (eds): Ultrasonic Examination of the Breast. New York, John Wiley & Sons, 1983, pp 379–384
7. Burns PN: Private communication
8. Wells PNT: Doppler studies for tissue characterization [Abstract]. J Ultrasound Med 3(suppl):134, 1984

9. Zlotecki R, Gyves J, Ensminger W: Epinephrine enhanced blood flow in rabbit hepatic VX2 implants. Proc Am Assoc Cancer Res 26:1392, 1985

10. Snyder R, Watson RC, Cruz N: Graphic stress telethermometry: A possible supplement to physical examination in screening for abnormalities in the female breast. Am J Diagn Gynecol Obstet 1:197, 1979

11. Jurist JM, Myers DB: Stress thermometry and breast disease: Does the computer make screening work? J Reprod Med 27:690, 1982

12. Weiner N: Norepinephrine, epinephrine, and the sympathomimetic amines. *In*: Goodman LS, Gilman AG (eds): The Pharmacological Basis of Therapeutics. New York, MacMillan, 1980, pp 144–148

13. Halliwell M: Private communication

14. Zlotecki R, Ullrich K, Ensminger W: Hepatic tumor vascularity in the rabbit VX2 tumor model [Abstract]. Proc Am Assoc Cancer Res, in press