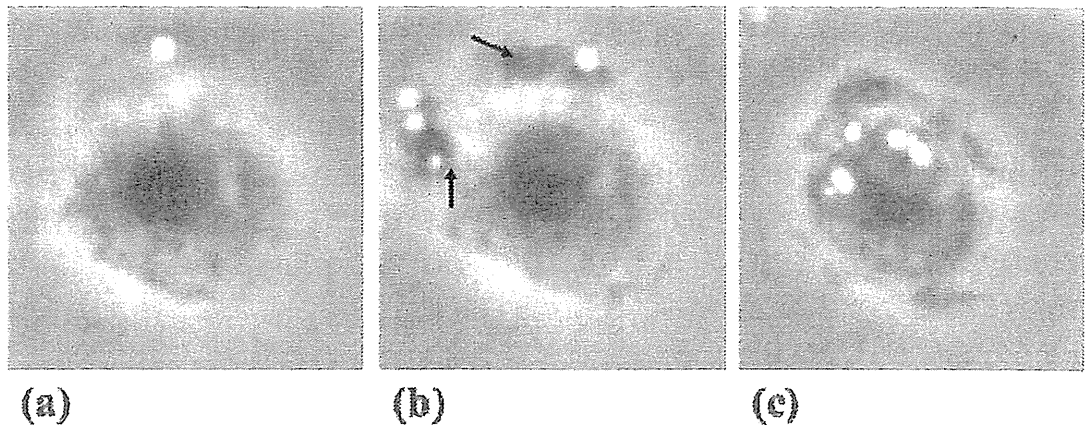

Figure 9.7 Microscopic photograph of phagocytosis of Sonazoid bubbles by cultured Kupffer cells. When the Sonazoid solution was poured into the culture medium of isolated Kupffer cells, bubbles were actively phagocytosed by the Kupffer cells. The Kupffer cells extended tentacles (arrows) to capture and phagocytose the bubbles. The phagocytosed bubbles became translocalised around the nucleus in approximately 10 min. During the translocalisation, the bubble diameter decreased and the bubbles became distorted in shape.

9.2.3 Diverse Effects of Sonazoid

9.2.3.1 Adverse effects of gas

Microbubbles are composed of gas, and gas embolism has been of concern as an adverse effect of ultrasound contrast agents. However, although next-generation contrast agents, including Sonazoid, have been administered more than 2 million times around the world, there have been no reports of embolic adverse effects. These cases include patients with right-to-left shunts such as patent foramen ovale; however, embolic adverse effects have never been reported. This is because microbubbles easily pass through the capillaries due to the small bubble diameter, the bubbles do not merge together and only a very small amount of gas is administered.

The gas content in the prepared Sonazoid solution is 1 vol%. Therefore, if one vial (2 mL) of Sonazoid solution is injected intravenously, the total amount of gas administered into the blood vessel is 20 μL. It can be said that even if all the gas coalesces to produce embolism, no adverse effects inducing organ dysfunction would be expected.

9.2.3.2 Adverse effects of the shell

As described above, the shell of microbubbles is composed of phosphatidylcholine from egg yolk. Therefore, the package insert states that "the agent should not be given to patients with egg allergy". However, although Sonazoid has been administered to more than 20,000 patients worldwide, including Japan, to date, there have been no reports of serious allergic adverse effects.

9.2.3.3 Physiological effects

If high acoustic power ultrasound is irradiated to the contrast agent incorporated into Kupffer cells (Fig. 9.7), the bubbles collapse, and in such a case, the physiological effects on the cells would be of concern. However, even if the contrast agent (Levovist or Sonazoid) incorporated into the liver is completely eliminated, no events, such as increase in blood LDH levels, have been found that are likely to reflect cell damage. In addition, theoretically, when the recommended dose is administered, one bubble is phagocytosed per 50–100 Kupffer cells. Therefore, even if the bubble collapses, cell necrosis or apoptosis will not be clinically observed as an adverse event despite some effect on the cells.

9.2.4 Administration of Sonazoid

9.2.4.1 Preparation of Sonazoid (Fig. 9.8)

Sonazoid, which is supplied as a lyophilised powder, is dissolved in 2 mL of distilled water to prepare the Sonazoid solution. As described above, the gas contained in the prepared Sonazoid bubbles is perfluorobutane, a fluorocarbon, (C_4F_{10}; common name, perflubutane). The gas contained in the vials both before and after preparation is perflubutane. If the vial caps are left open, the gas will be replaced by air and gas exchange would occur between the gas in the bubbles and air, leading to a reduction in the contrast effect of Sonazoid due to the gradual replacement of the gas in the bubbles by air. Similarly, if degassed water is not used to dissolve Sonazoid, the perflubutane in the bubbles will be replaced by nitrogen and other gases dissolved in water. Therefore, degassed distilled water for injection must be used to dissolve Sonazoid.

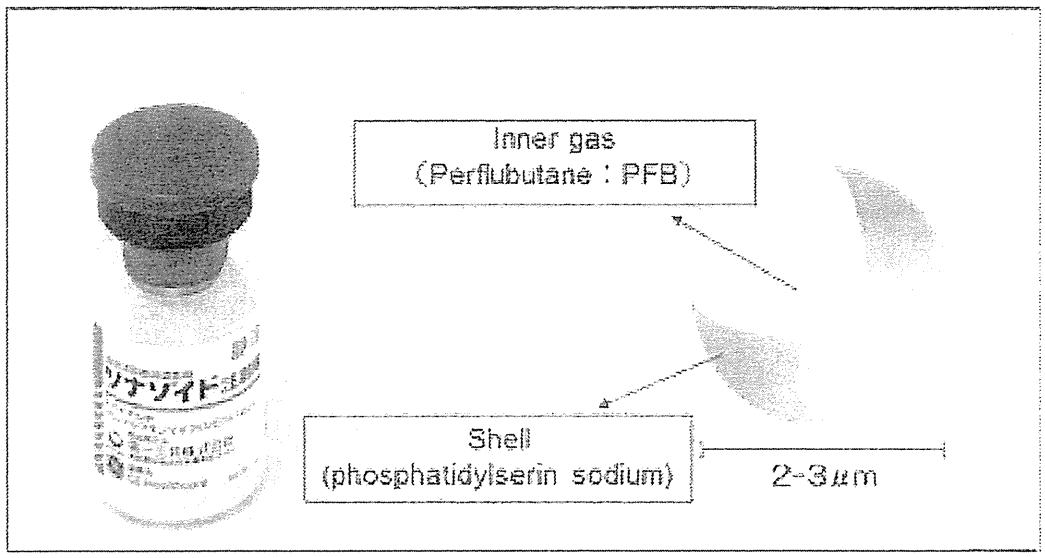

Figure 9.8 Schema for the Sonazoid vial and microbubbles. The lyophilised powder of Sonazoid is dissolved in 2 mL of distilled water. The gas in the vial is perfluorobutane; therefore, conditions should be avoided in which the gas in the upper solution is replaced by air, such as leaving the cap of the vial open, after preparation of the solution. Even after dissolving the Sonazoid and preparing the solution, gas exchange occurs between the gas in the bubbles and air outside the solution.

Sonazoid is usually administered by intravenous bolus injection into a peripheral blood vessel. An elbow vein is selected to increase the peak blood concentration after intravenous injection. The intravenous injection volume of Sonazoid solution is as small as ≤1 mL, and the peak blood concentration can be increased by flushing with 10–20 mL of physiological saline.

Usually, an extension tube filled with the Sonazoid solution is used, and after flushing with 10–20 mL of physiological saline, the intravenous bolus injection is given. At this time, high pressure should not be applied to the Sonazoid bubbles in the extension tube. If pressure above a threshold is applied, the bubbles in the tube will disappear within a moment.

9.2.4.2 Dose of Sonazoid

The package insert states that the recommended dose of Sonazoid is 0.015 mL/kg body weight. Therefore, the recommended dose is 0.9 mL in adults with a body weight of 60 kg. However, when using a sensitive harmonic imaging method, a good contrast effect can be obtained even with half the dose. At our institution, a dose of 0.5 mL

is used, irrespective of the body weight. Four injections can be given per vial.

9.2.5 Apparatus

9.2.5.1 Ultrasonic diagnostic apparatus suitable for imaging

An ultrasonic diagnostic apparatus with an imaging mode suitable for the imaging is used. Basically, nonlinear imaging methods (harmonic methods) are used. In the newly developed apparatus, tissue harmonics of the phase modulation method are often used in the usual non-enhanced B-mode, and these are used for imaging by reducing the transmission acoustic power and number of frames.

The conventional fundamental-wave B-mode can also be used for imaging; however, the quality of imaging is worse in this mode than in the harmonic mode. The quality of imaging is improved by increasing the contrast agent dose; however, an overdose would cause shadowing, resulting in a worse contrast effect.

9.2.5.2 Moving and still image recording

Moving images obtained are recorded as moving and still images. Dynamic studies are recorded for 3 min, and moving Kupffer images are recorded for 10–15 s by scanning a single window. It is important to carry our diagnosis on moving images, and it is expected that the moving images obtained can be sent to a reading room and diagnosed on a monitor by radiologists in the near future.

9.2.6 Imaging Modes

9.2.6.1 Principles

9.2.6.1.1 Harmonic imaging method

Ultrasound images are obtained by irradiating ultrasound and visualising the ultrasound reflected by the scatterers in living bodies. Living tissues produce strong linear scattering, while microbubbles produce strong non-linear scattering; namely, the waveform of the irradiated ultrasound is different from that of the ultrasound scattered by the microbubbles. This non-linear scattering shows a frequency distribution with peaks at integral multiples of the

frequency of the irradiated ultrasound. For example, if ultrasound with a frequency of 2 MHz is irradiated, ultrasound with peak frequencies of twice (4 MHz) and three times (6 MHz) the irradiated frequency is reflected. Components at twice or three times the fundamental frequency are called the second and third harmonics, respectively. Furthermore, ultrasound with a peak frequency of one-half (1 MHz) is also reflected, which is termed a subharmonic.

The second harmonic is well understood as a signal from the bubbles and used in images. In the frequency distribution, the ultrasound generated by the resonance of bubbles shows high peaks, but when the bubbles are collapsed by a stronger ultrasound, the valleys are filled.

The phase and amplitude modulation methods described below use the second harmonic and harmonic components of the fundamental wave, respectively, for visualisation.

9.2.6.1.2 Phase Modulation (PM) and Amplitude Modulation (AM) methods (Fig. 9.9a,b)

In the phase modulation (PM) method, two pulse waves with a phase difference of 180° are transmitted, and the two scattered waves received are added together for visualisation. There are differences in the degree of distortion of the waveform between ultrasonic waves scattered from tissues and from bubbles. Better contrast sensitivity is obtained with bubbles than with living tissues due to the more non-linear components.

On the other hand, in the amplitude modulation (AM) method, two pulse waves with the same phase and different amplitudes (for example, equal amplitude and one-half amplitude) are transmitted and the wave obtained by subtracting the doubled reflected wave of the one-half amplitude wave from the reflected wave of equal amplitude is visualised.

The PM and AM methods differ in that the spatial resolution is better in the PM method and the sensitivity in deeper areas is better in the AM method. In particular, when relatively high acoustic power is used instead of low acoustic power, like in Sonazoid, non-linear signals from the tissues (tissue harmonics) are strong. Therefore, methods, such as the AM method, that can suppress tissue harmonics are effective for increasing the bubble-tissue ratio in signals.

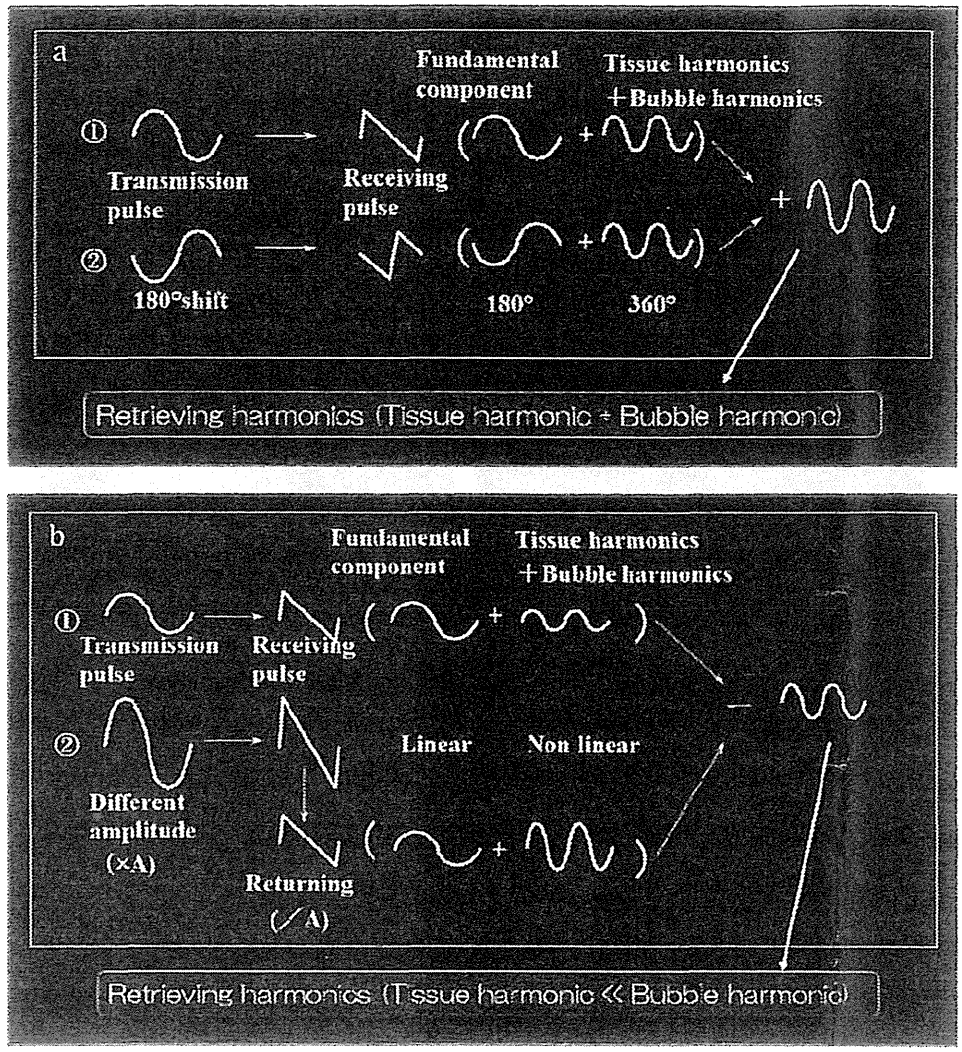

Figure 9.9 a Principle of the phase modulation method. In this method, two pulse waves with a phase difference of 180° are transmitted and received to extract nonlinear components scattered by tissue and bubbles. This method is characterised by the ability to extract the second harmonics, with twice the frequency, from both tissues and bubbles. b Principle of the amplitude modulation method. In this method, two pulse waves with different amplitudes are transmitted and received to extract nonlinear components scattered by tissue and bubbles. This method is characterised by the ability to extract nonlinear components of equal amplitude (fundamental wave band), mainly from bubble harmonics.

9.2.6.2 Imaging condition

9.2.6.2.1 Acoustic power (Fig. 9.10)

Among the imaging conditions, acoustic power is the most important. Next-generation ultrasound contrast agents are generally said to be low-acoustic-power contrast agents; however, the optimum acoustic power varies somewhat among contrast agents.

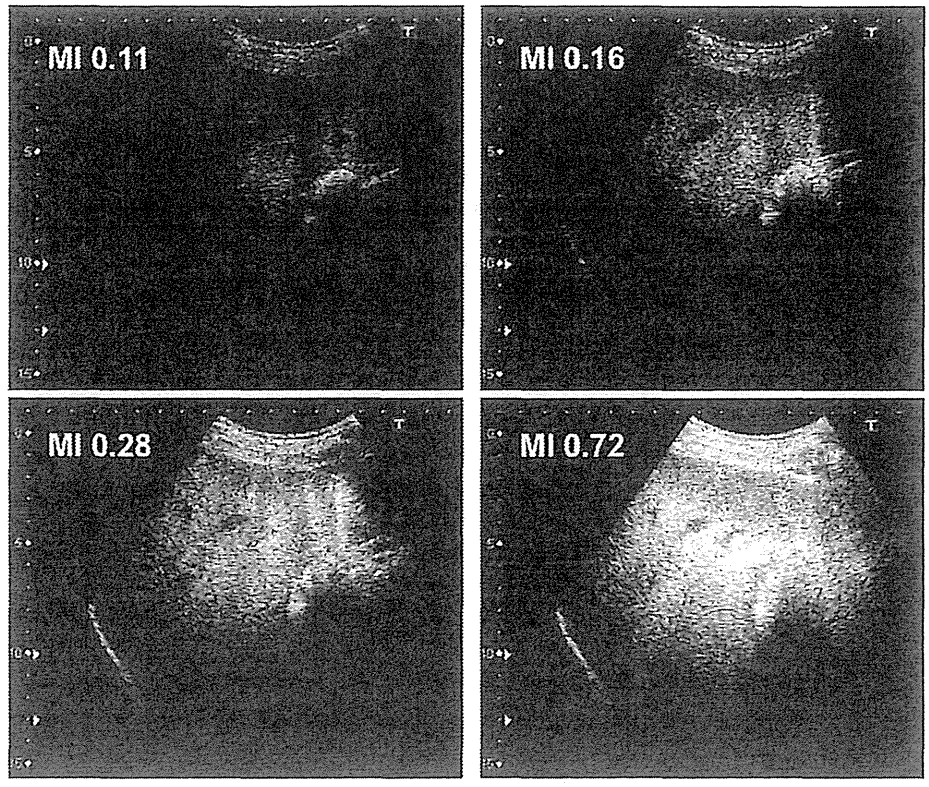

Figure 9.10 The quality of imaging according to the acoustic power setting. When the acoustic power was changed 10 min after injection of 0.5 mL of Sonazoid, the imaged areas expanded with increase in the acoustic power. However, at an MI of 0.72, the bubbles disappeared and signals became attenuated near the surface of the liver. Thus, the resonance and destruction of bubbles strongly depend on the acoustic power.

Vibrating acoustic power and destroying acoustic power: When acoustic power is gradually increased in living bodies containing bubbles, the state in which the bubbles are not vibrated or destroyed is changed to the state in which the bubbles begin to vibrate. When the acoustic power is further increased, the bubbles are destroyed in a short time. The acoustic power required to destroy bubbles varies among contrast agents, and the destroying acoustic power is highest in Optison, followed by SonoVue, Imagent, Definity and Sonazoid, in that order. In the case of Sonazoid, the bubbles begin to collapse at an MI of more than 0.4.

9.2.6.2.2 Particle size distribution of the bubbles and acoustic power

Ultrasound contrast agents composed of microbubbles, such as Sonazoid, have a characteristic distribution of bubble diameters. It is said that Sonazoid has a narrower particle size distribution (higher homogeneity) than other ultrasound contrast agents.

In general, the smaller the bubble diameter, the higher the acoustic power required to produce vibration and destruction of the bubbles. For example, after transmission at an MI of 0.5 to obtain images, when the MI is increased to 1.0, the bubbles that were not destroyed at an MI of 0.5 were destroyed and generated signals.

In addition, the spleen is also a reticuloendothelial organ, and bubbles accumulate in the spleen over 10 min after intravenous injection of Sonazoid. It is known that the size of the particles incorporated into the macrophages in the spleen is smaller than that of the particles incorporated into the Kupffer cells in the liver. As a result, sonazoid accumulated in the spleen is less susceptible to destruction than that accumulated in the liver.

9.2.6.2.3 Bubbles circulating in the blood and bubbles within cells

Sonazoid bubbles remain in the circulating blood after intravenous injection, but are phagocytosed by macrophages throughout the whole body with time, except those that naturally collapse. When looking at the process of phagocytosis of Sonazoid bubbles by cultured Kupffer cells, the bubbles are incorporated into the cytoplasm from the cell surface and move towards the nucleus (Fig. 9.7); this process takes a few to 20 min.

Bubbles in the cells are less susceptible to vibration and destruction than bubbles in the circulating blood. This is because the viscosity of the cytoplasm is higher than that of the blood, and bubbles in the cells are covered by a membrane. In addition, another reason is that after being phagocytosed by the Kupffer cells, the Sonazoid bubbles become smaller than the extracellular bubbles. Therefore, higher acoustic power is required to obtain Kupffer images than to obtain vascular images.

9.2.6.2.4 Number of frames

Sonazoid imaging is characterised by real-time images. The susceptibility to destruction varies according to the number of frames per unit time (frame rate, fps), even under the same acoustic power, and the greater the number of frames (frame rate), the greater the susceptibility to destruction. Particularly in the focus area, the acoustic power is high and beams overlap in each transmission at short distances, causing destruction of bubbles.

Therefore, the quality of the imaging is worse than that in other areas.

It is necessary to obtain images with the minimum number of frames while maintaining real-time imaging, and approximately 15 frames/s (fps) is usually recommended.

9.2.6.2.5 Focus (Fig. 9.11)

In the focus area, acoustic power is high, and bubbles only in this area are vibrated or destroyed, producing heterogeneous liver images. To avoid this, multistage focusing and depth-independent beam forming are used. When a single focus is used, it is necessary to find the appropriate focus setting, such as focusing on the deepest area.

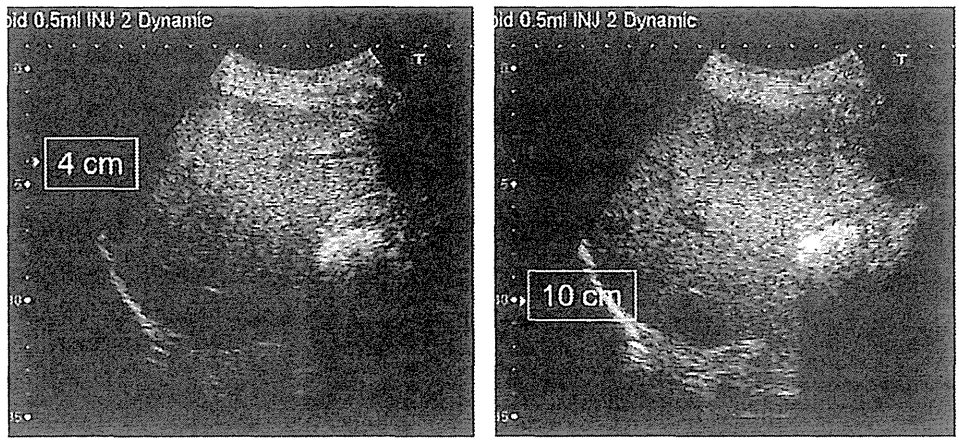

Figure 9.11 Changes in the quality of imaging according to the focus. The focus depth is 4 cm in the left panel and 10 cm in the right panel. As the focus became deeper, bubbles from deeper areas began to vibrate and images could be obtained. Images obtained 10 min after injection of 0.5 mL of Sonazoid are shown.

9.2.7 Actual Image Reading

9.2.7.1 Dynamic study (Figs. 9.12–9.14)

After intravenous injection of Sonazoid, real-time imaging of the liver can be performed and recorded. Usually, the hepatic artery is imaged in 15–20s, followed by the portal vein. The portal vein is predominantly imaged between 20 and 30s, and after 30s, the equilibrium phase is reached, in which the artery and portal vein are similarly imaged. This equilibrium phase is recorded until 3 min after the intravenous injection. Thereafter, Kupffer images become predominant.

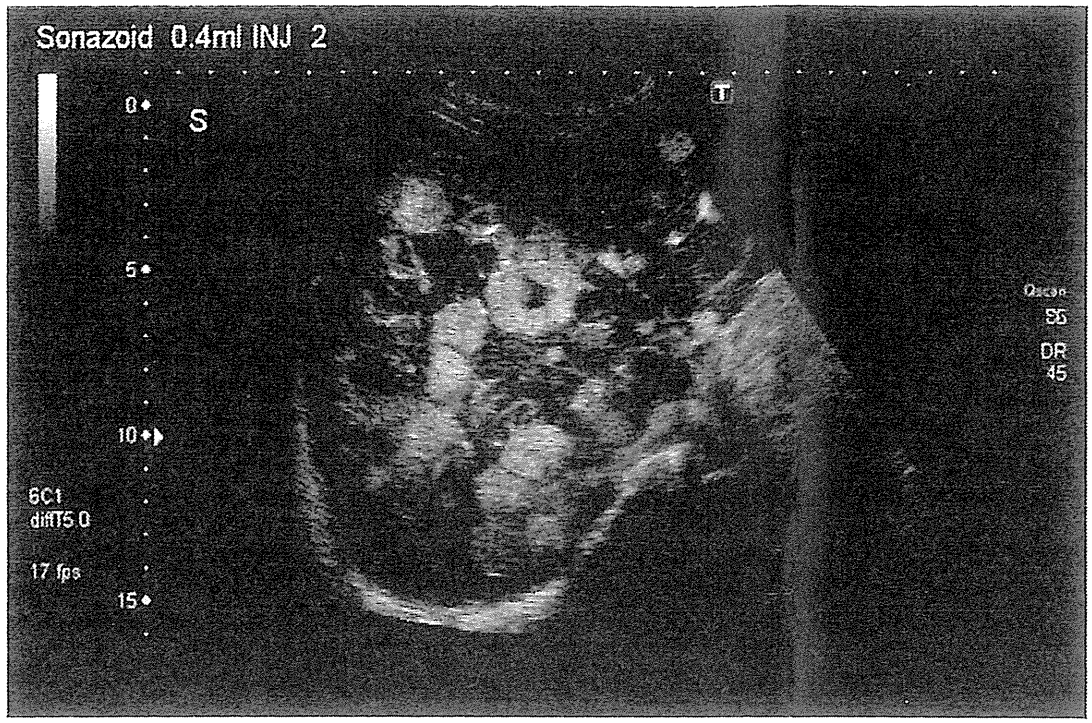

Figure 9.12 Sonazoid images of HCC. The arterial phase after intravenous injection of 0.4 mL of Sonazoid is shown. Many tumours with numerous densely stained arteries are seen in the liver.

9.2.7.2 Flash Replenishment Imaging (FRI) and Micro-Flow Imaging (MFI) (Fig. 9.15, 9.16)

If transmission is made at a high acoustic power (MI ≥ 1.0) and 10–30 frames are scanned when the parenchyma is filled with the contrast agent, bubbles in the scan volume are totally destroyed. Thereafter, low acoustic power contrast harmonic imaging can be used to visualise the reperfusion of blood containing new bubbles in the scan volume under low acoustic power. This imaging of reperfusion is called flash replenishment imaging (FRI; Fig. 9.15, 9.16).

Furthermore, when visualising the reperfusion after destruction of bubbles with FRI, fine blood vessels can be continuously visualised by adopting the maximum intensity holding technique, which maintains the maximum intensity in each pixel. This method is called micro-flow imaging (MFI; Fig. 9.15, 9.17–9.19).

MFI, as well as FRI, can be repeatedly performed at any time phase in any part of the liver. In MFI in the arterial phase, tumour vessels that control arterial blood flow are more clearly depicted than non-tumour vessels. In addition, in the portal venous predominant and equilibrium phases, both the arterial and portal venous blood