

Ultrasonographic Manifestations of Liver Abscesses; An Experimental Study

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ABSTRACT

The livers and portal veins of 3.0–3.5 kg male rabbits were inoculated with *S. aureus*, and *E. coli* combined with *B. fragilis* to induce liver abscesses and relate ultrasonographic images to pathologic findings. There were no morphological differences between those of *S. aureus* and the anaerobic infections. Small abscesses were generally echogenic, and consisted histologically of polymorphs, coagulation and granulation tissue. These irregularly-margined structures with liquefactive necrosis had “bull’s-eye” appearances. So-called “established abscesses” with hypoechoic surroundings were also observed, with central liquefactive necrosis, polymorphs and granulation tissue peripherally. The peripheral echo-free halos were attributed to coagulation necrosis.

Key words: Liver abscess, Ultrasound studies

So-called “established abscesses” are relatively easy to diagnose ultrasonographically; i. e., by their irregular, thick walls, low internal echoes and accompanying posterior echoes^{3,8,12}). However, early liver abscesses are difficult to detect and have variable sonographic appearances^{2,16}). Furthermore, no correlative pathological studies have been performed relative to ultrasonographic images of early liver abscesses. In this study liver abscesses were experimentally-induced and comparatively investigated to establish correlations between ultrasonographic images and the histopathology of acute liver abscesses. The effect of the type of causative bacterium or infection pathway on the liver abscess morphology, the image produced by the early liver abscess, and the natural history of the liver abscess, were investigated.

Table 1

Inoculation site	control	<i>S. aureus</i>	<i>E. coli</i> + <i>B. fragilis</i>
Liver	Group 1	Group 2, 3	Group 5
Portal vein		Group 4	Group 6

MATERIALS AND METHODS

Domestic rabbits weighing 3-3.5 kg were categorized in 6 groups according to inoculation site, bacterial type, and bacterial quantity (Table 1). Ultrasonographically-guided inoculations of the left lobes of their livers, or the umbilical portions of

their portal veins were performed, because the lateral segment is the largest segment in the rabbit's liver and the umbilical portion of the liver is also prominent. Suspensions of each bacterial species combined with quantities of gelfoam, usually about 20 pieces, each 1 mm square, based on rabbit weights, were inoculated. The viable cells of *Staphylococcus aureus* FDA 209P JC-1 (*S. aureus*) were approximately $3-5 \times 10^{10}$ CFU and for *Escherichia coli* NIHJ-JC-2 (*E. coli*) approximately 1.5×10^8 CFU in combination with approximately $3-5 \times 10^{10}$ CFU of *Bacteroides fragilis* ATCC 25285 (*B. fragilis*). Intramuscularly, steroid (Solu-Cortef 250 mg, Upjohn Japan) was injected twice weekly for 2 weeks to promote abscess formation¹⁾ and intravenous injections of cyclophosphamide (Endoxan 20 mg, Shionogi Japan) were administered weekly for 2 weeks for immunosuppression except in group 2. For each control rabbit, 1.5 ml physiological saline in combination with gelfoam were intrahepatically inoculated. Rabbits in group 2 were sacrificed 1, 2, 3, 5, 7, and 14 days postinoculation and those in the other groups were sacrificed 2, 3, 5 days postinoculation corresponding to group 2. Extension from intraperitoneal and subcapsular abscesses were included in subjects. Intracystic and aural vein inoculations were also studied, but they were later excluded from study because they did not induce large abscesses. Bacterial counts (in colony-forming units; CFU) were ascertained using suspensions prepared from cultures of *S. aureus* or

Table 2. Incidence of liver abscesses

Group	1 n=5	2 n=20	3 n=10	4 n=6	5 n=10	6 n=5
not detected if less than 3 mm or isoechoic (3 ± 1 mm) echogenic 5 mm average (5 ± 2 mm) target appearance 9 mm average (9 ± 2 mm) established abscess more than 10 mm (15 ± 5 mm)	5	13	3	3	1	4
		6	3	1	1	
		1	1	1	6	
			3		2	1
rate (%)	0	35	70	17	90	20

E. coli in a brain heart infusion (BHI) medium, and of *B. fragilis* in a GAM medium (Nissui Seiyaku Co., Japan).

Ultrasonographic scans were performed everyday in the living subject using a real time apparatus and 5 MHz transducer, and a water bath for necropsy. Liver tissues were prepared using H & E, Azan and Gomori stains.

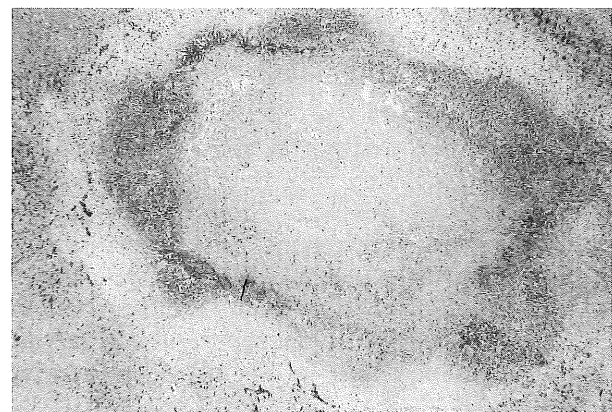
RESULTS

The incidence of liver abscess formation is shown in Table 2. Three rabbits died from intraperitoneal bleeding after inoculation not, from the umbilical portion, but from the main portal vein; two were in group 4 and one was in group 6. In the echogenic case in group 4, abscesses were not observed, but portal thrombosis and congestion of the affected liver were observed. Immediately after intraportal inoculation, air-containing gelfoam was observed to be echogenic, and scattered peripherally in the left lobe. However, it was not detected a few minutes thereafter. In the cases of intrahepatic inoculation, gelfoam was scattered in the liver or the hepatic fissure, and it was not detected 1 day postinoculation. In members of the control group, which received gelfoam and saline solutions, most of the gelfoam did not remain within the liver. Only a few gelfoam pieces remained within the liver; nearly all gelfoam fragments were surrounded by liver capsule or the greater omentum.

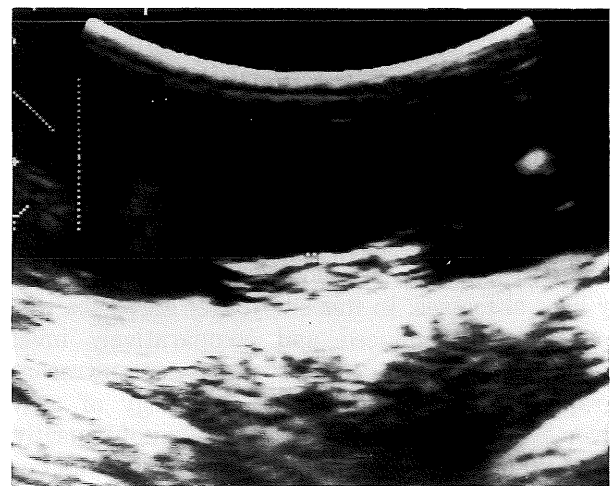
In groups receiving intrahepatic inoculations: ultrasonography revealed few abscesses, even with little CFU such as in Group 2 (10^8 CFU of *S. aureus*), but they were smaller than those detected in the other group receiving steroid and cyclophosphamide injections.

Anaerobic infections tended to induce abscesses larger than those induced by *S. aureus* infections. With the combined inoculation of *E. coli*, and *B. fragilis*, *B. fragilis* caused abscesses, as previously reported^{5,7}.

The ultrasonographic images of hepatic abscesses induced by intrahepatic inoculations were categorized as a) echogenic images, b) classical hypoechoic images with hyperechoic margins, c) "bull's eye" or "target" images, and d) no detectable images. It was impossible to obtain an image from an ab-



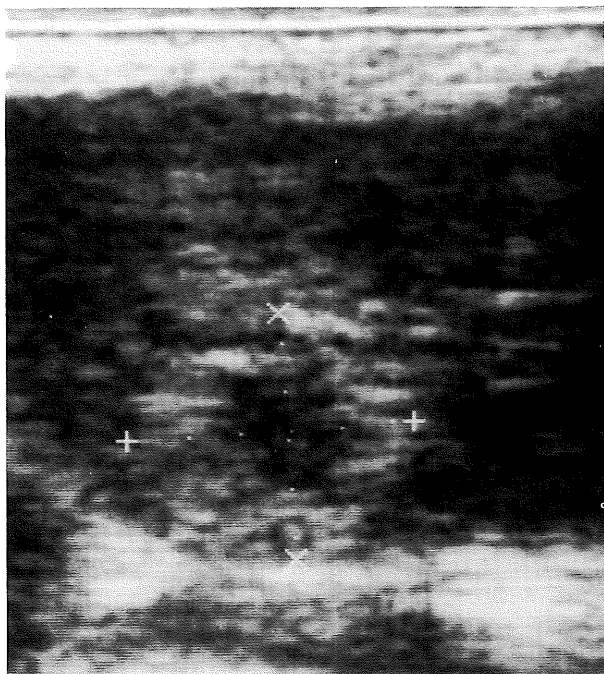
a



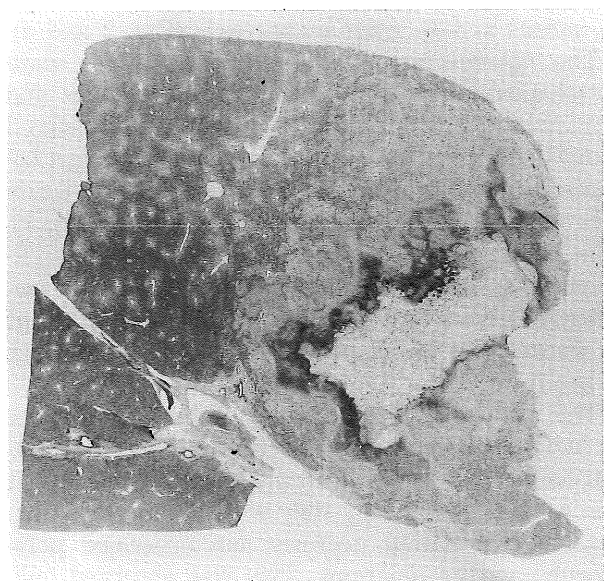
b

Fig. 1. Four days after liver inoculation (Group 2) a. Coagulation necrosis and inflammatory cells surrounded by granulation tissue (H & E stain $\times 4$). b. Sonogram shows an echogenic lesion.

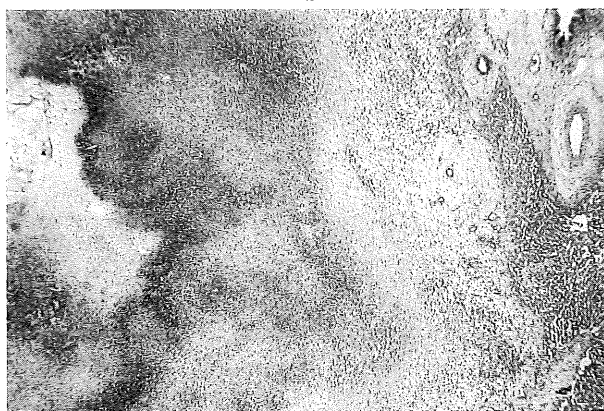
cess whose diameter was 3 mm or less. Small abscesses were echogenic (Fig. 1). They appeared as 3-layered structures of polymorphonuclear infiltrates, coagulation necrosis, and granulation tissue, and their mean diameter was 5 mm. "Established abscesses" had diameters of at least 10 mm, and contained liquefactive necrosis surrounded by polymorphs and granulations. Abscess walls which were echogenic were composed of polymorphs and granulations (Fig. 2). Abscesses exhibiting "bull's-eye" or "target"-like images apparently



a

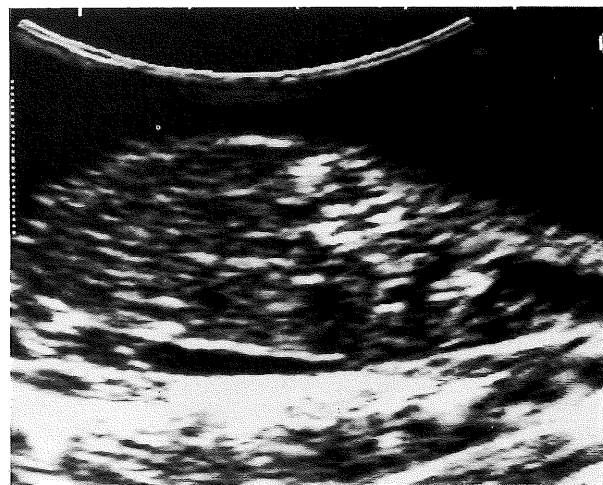


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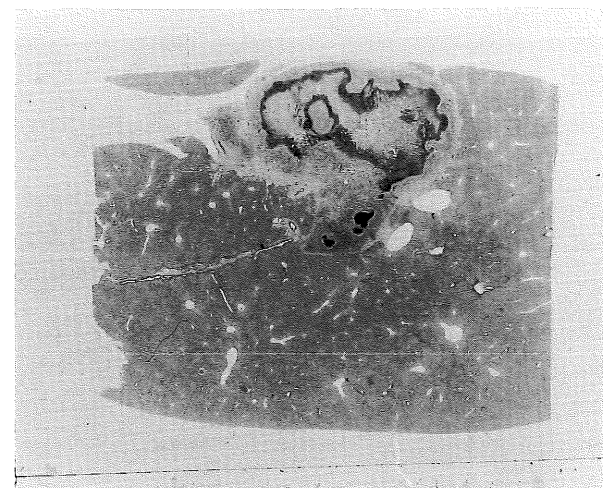


c

Fig. 2. Five days after liver inoculation (Group 3)
 a. Hypochoic and echogenic surroundings were observed in the so-called established abscess.
 b. The corresponding macroscopic slice.
 c. Liquefactive necrosis is surrounded by inflammatory cells and granulations (H & E stain $\times 4$).



a



b



c

Fig. 3. Fourteen days after liver inoculation (Group 5)
 a. Increased echogenicity observed in the periphery and centrally resembled a target image.
 b. The macroscopic slice
 c. Coagulation necrosis, inflammatory cells and granulations composed the abscess (H & E stain $\times 4$).

were 3-layered structures consisting of coagulation necrosis, polymorphs, and granulations, but their margins were irregular (Fig. 3). The mean diameter of these abscesses was 9 mm. Distal acoustic enhancement was not observed unless coagulation

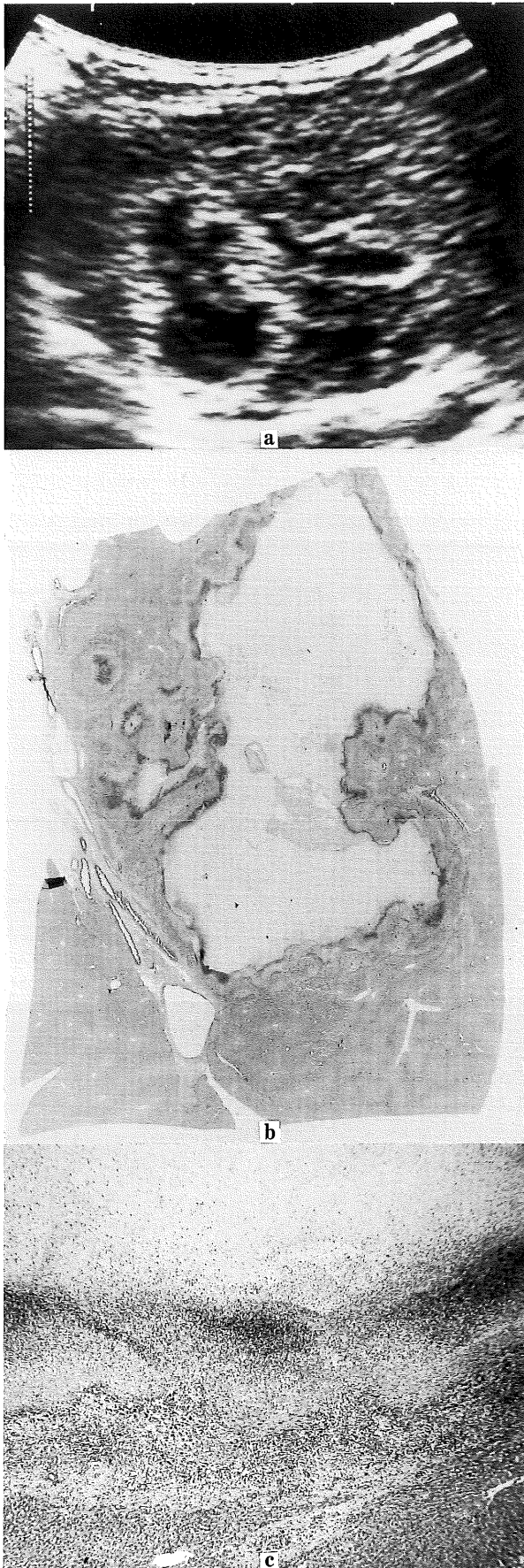


Fig. 4. Seven days after liver inoculation (Group 5)
 a. Oblique scan showing an established abscess.
 b. The corresponding macroscopic slice.
 c. Liquefactive necrosis surrounded by inflammatory cells and granulation tissue (H & E stain $\times 10$).

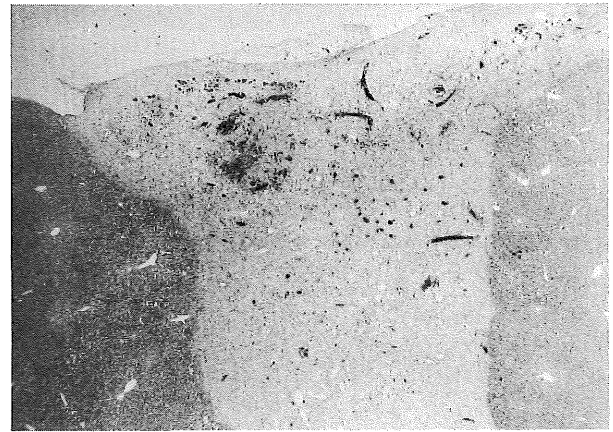


Fig. 5. Fourteen days after liver inoculation (Group 2) Fibrosis was beginning to appear and foreign-body granulomas remained within the granulation (H & E stain $\times 2$)

necrosis became liquefactive necrosis.

There were no morphological differences between *S. aureus* and *B. fragilis* abscesses (Fig. 2 and 4).

The natural histories of acute abscesses were histologically confirmed. Coagulation necrosis and accumulations of inflammatory cells were observed 2 days post-inoculation. The coagulation necrosis became liquefactive necrosis (Fig. 2) and granulation was apparent a few days post-inoculation (Fig. 1). One week post-inoculation in small abscesses, fibrous tissue surrounded the granulations and calcifications were among the granulations. And about 2 weeks post-inoculation, fibrosis was apparent (Fig. 5).

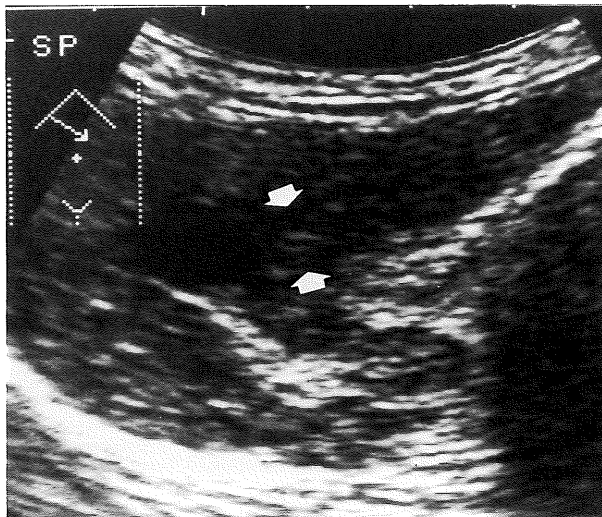
The ultrasonographic images of hepatic abscesses induced by intraportal inoculations were indistinguishable from those of normal parenchyma except one case. Histologically they were composed of extensive coagulation necrosis, circumscribed polymorphs and granulations. This was observed only in rabbits which received intraportal inoculations, and it was not liable to soften and liquefy (Fig. 6). In one longstanding case, granulation and fibrosis surrounded coagulation necrosis, causing a "bull's-eye" image (Fig. 7). Table 3 summarized sonographic findings versus histologic correlation.

Table 3. Ultrasonographic-histologic comparison

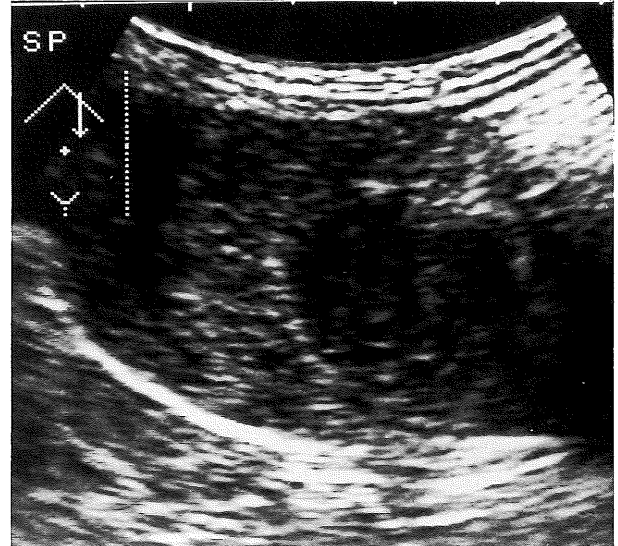
Granulation Tissue	Fibrosis	Coagulation Necrosis	Liquefactive Necrosis
Hyperechoic but gradually decreasing echo	Hypoechoic	Isoechoic or hypoechoic solid	Hypoechoic fluid

DISCUSSION

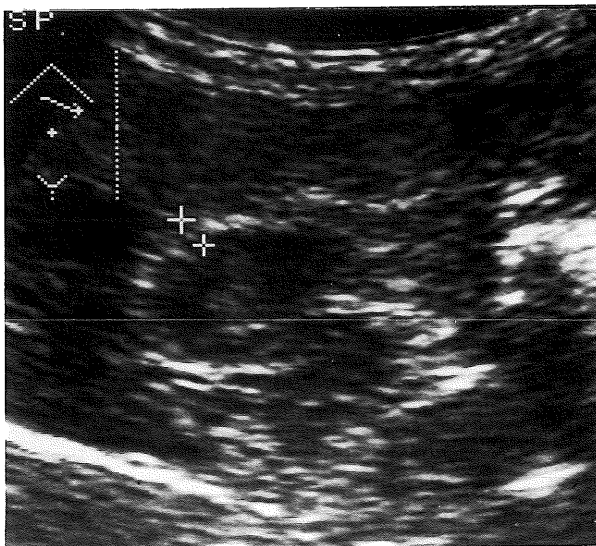
A previously reported method of inducing abscesses experimentally consisted of inoculating a bacterial suspensions into ligated common ducts⁷⁾,



a



c



b



d

Fig. 6. Group 6

a. Sonogram two days after portal vein inoculation showed echo-free halo (arrow).

b. Scan two days later, showed diminution of echo centrally and echogenic surroundings, and parenchymal echoes were comparatively echogenic with hepatomegaly.

Fig. 6

c. Sixteen days after inoculation, echogenic surroundings became thinner than in image b and the central low echo became slightly lower than normal liver.

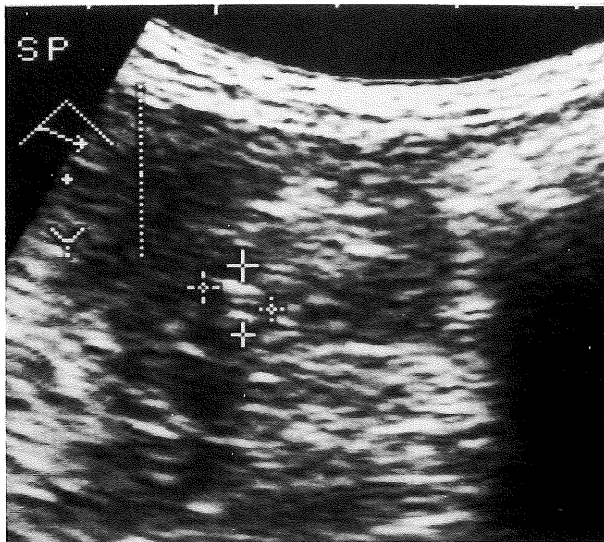
d. The corresponding macroscopic slice showing a large zone coagulation necrosis surrounded by polymorphs and granulation tissue.

but this was not suitable for the present study because it involved an operational procedure and repeated ultrasonography. Therefore, intrahepatic gelfoam inoculations were used to induce liver abscesses.

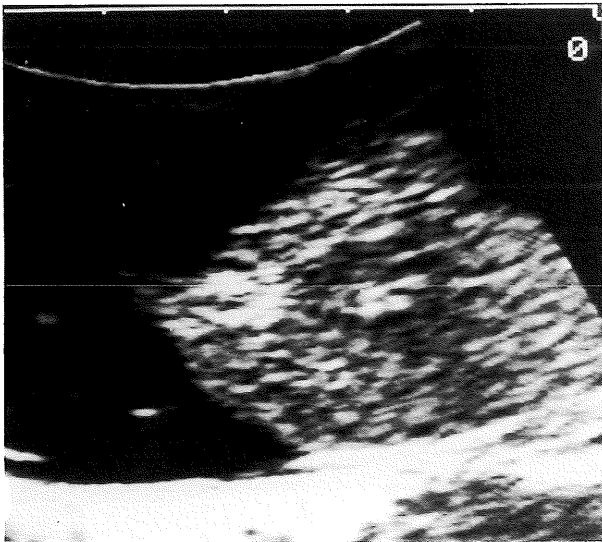
In rabbits in which abscesses formed, retained gelfoam was observed both in isoechoic and hypoechoic sites. Based on this, it was concluded that gelfoam does not influence the echogenicity of an abscess.

Avenues of infection included the portal vein, hepatic artery, biliary tract and direct extension from an adjacent infected organ^{6,15}. Intracystic, intravenous and intraportal inoculations were performed, but satisfactory abscess formation occurred only in intraportal inoculation.

By intrahepatic or intraportal inoculation, there were no morphological differences in the abscesses according to the causative bacteria. However, abscesses whose extensive coagulation necrosis was surrounded by polymorphic infiltrates were observed only in a case in which intraportal inoculation was used. Inversely, this suggests that abscesses with relatively uniform internal echoes are induced by portal infections. And they resembled pathologically the infarct of Zahn circumscribed with inflammatory cells, and resembled ultrasonographically amoebic liver abscesses which were hypoechoic relative to normal liver parenchyma, and had fine, homogeneous, low-level echoes¹³. Coagulation necrosis was always nearly isoechoic or hypoechoic relative to normal liver



a



b

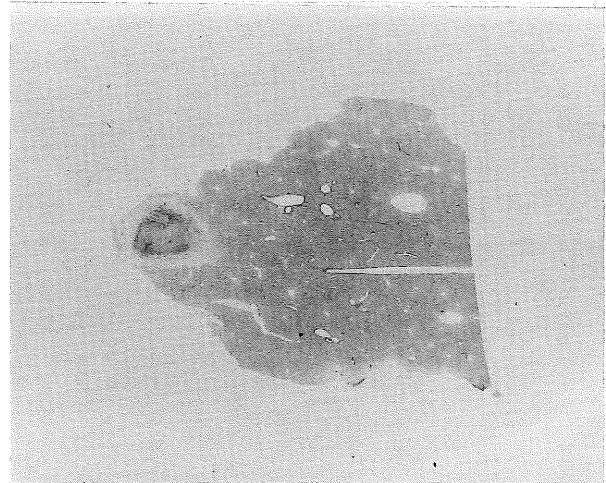
Fig. 7. Twenty-one days after portal vein inoculation (Group 4)

a. An ultrasound scan *in vivo* showing a bull's eye image.

b. *In vitro* image.

parenchyma; it was never hyperechoic. On that ground, coagulation necrosis does not take part in the hyperecho of abscess wall. The morphological differences between intrahepatic and intraportal inoculations may be due to the pattern of inflammatory cells in the abscesses. In the case of intrahepatic inoculation, inflammatory cells were concentrated inside the abscess, but in the case of intraportal inoculation, they were not observed in the center of the coagulation necrosis, but surrounded the periphery. Therefore secondary liquefactive necrosis may not occur by the proteolytic enzymes from neutrophils and the coagulation necrosis may not soften and liquefy.

The natural history of liver abscesses indicates that an early abscess can be defined as that which has no granulation and/or whose coagulation necro-



c



d

Fig. 7

c. The corresponding macroscopic slice.

d. Histologically, granulation and fibrosis surrounds coagulation necrosis (H & E stain $\times 4$).

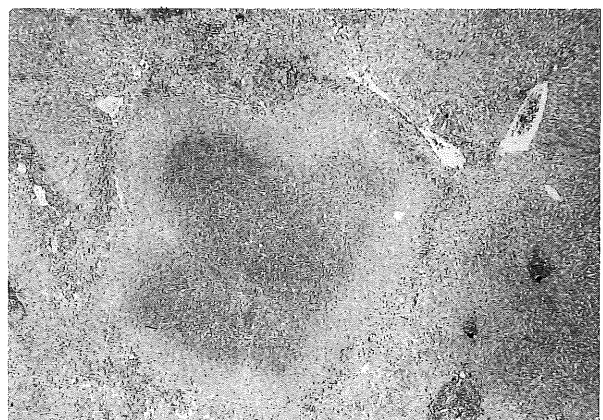


Fig. 8. Two days after inoculation (Group 2)

Accumulation of inflammatory cells. Coagulation necrosis is in direct contact with the liver cells (H & E stain $\times 4$)

sis progresses to a liquefactive necrosis. Coagulation necrosis was in direct contact with liver cells and accumulation of inflammatory cells were within the coagulation necrosis within 2 days of inocula-

tion ("hyperacute phase") (Fig. 8). The spectrum of ultrasonographic manifestations of liver abscesses in the hyperacute and acute stages differs with the echogenicity of coagulation necrosis, with increases in accumulations of inflammatory cells, and with abscess sizes. Their contents are continually changing during their phases of development, organization, and repair¹⁶. Variable reported ultrasonographic appearances of abscesses have been entirely of this phase and these stages^{2,4,9,10}. If the effects of bacterial toxins are severe, the liver cells or granulation tissue surrounding the abscess may become necrotic like a hyperacute phase of an abscess and the abscess may infiltrate its surroundings. The coagulation necrosis which surrounds the abscess in the hyperacute phase is regarded causative of the peripheral echo-free "halo". Follow-up scans showed that the ring is eventually incorporated into the abscess cavity (Fig. 6)². Edema or liver cell compression was not considered essential to cause the "halo"^{8,11}.

The echogenic wall consisted of granulation tissue and polymorphs. The echogenicity of granulation tissue may be due to numerous newly-formed blood vessels. Granulation tissue gradually changes to collagen tissue, and capillaries and inflammatory cells disappear. In longstanding cases, the thickness of granulation (Fig. 6), and the thickness of the halo of the "target" image (Fig. 7) show that the echogenicity of granulations decreased with age. "Target" images are regarded small "established abscesses"¹⁴.

In conclusion, liver abscesses were experimentally-induced via either intrahepatic or intraportal inoculations. No morphological differences were noted among causative bacteria, but there were differences among the respective infection pathways. With intrahepatic inoculations, coagulation necrosis rapidly became liquefactive necrosis; with intraportal inoculations, coagulation necrosis was not likely to soften and liquefy. Echogenic walls were mainly due to infiltrations of inflammatory cells and granulation. Halos observed in early abscesses are regarded attributable to coagulation necrosis.

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