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# Quantitative Evaluation of Ultrasonic Attenuation in Diffuse Liver Disease-Carbon Tetrachloride Induced Fatty Liver[1]

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=Abstract=The objective of tissue characterization is to provide quantitative information about the physical state of soft tissue interrogated by an ultrasound beam. The approaches adopted include measurement of ultrasonic attenuation, assessment of scattering properties and recently measurement of sound speed from the image shift.

In this experimental study of the ultrasonic attenuation process, the authors produced fatty livers in adult Wistar rats by the intraperitoneal injection of the toxic agent carbon tetrachloride(CC14) and measured the tissue characteristic parameter-the attenuation coefficient slope.

The results demonstrate that (a) the attenuation coefficient slope in the liver can be used to assign and quantify the state of diffuse liver disease, especially the degree of fatty changes (b) frequency dependence of attenuation reveals utility of this method over the routine study of tissue structure.

Key Words: ***Tissue characterization, Liver, Attenuation, Diffuse disease, Spectral analsis, Pulse-echo method, Transfer function***

## INTRODUCTION

While ultrasonography has become indispensable for imaging gross anatomy of internal organs, conventional technology is limited because of the loss of information inherent in the present systems.

There are two reasons for this;

(a) failure to take into account the frequency and phase characteristics of the ultrasonic echo signals.

(b) lack of attention to the characteristics of the transducer and electronic circuits during the signal processing and image reconstruction.

To realize and make use of the vast potential of diagnostic ultrasound, these deficiencies need to be overcome by new techniques and developments. One of such fields is ultrasonic tissue characterization.

The objctive of research into tissue characterization is to provide new ultrasonic parameters that can be used to measure the physical state of tissue.

Early attention was given to ultrasonic attenuation changes in myocardial infarction and ophthalmological tissues, and recent work has included organs such as the liver and breast(Garra *et al.* 1987).

Although the early in-vitro results have shown the possibility for the clinical application of attenuation parameters, they are developing slowly because of the technological and theoretical reasons. Clinical measurements of the true ultrasonic attenuation coefficients of tissue or of a slope parameter related to attenuation require the optimal design of hardware, and signal processing algorithms based on the complex acoustic theory.

Further, interactions between the ultrasound waves and the tissue being examined are poorly understood at this time (King *et al.* 1985).

In this paper, the authors studied ultrasonic attenuation coefficient slopes of rat livers affected by carbon tetrachloride($CCl_4$) poisoning. Carbon tetrachloride toxicity has been widely studied in the past few decades, and is known to affect the liver biochemistry and structure, leading to a condition

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characterized as "fatty liver", analogous to fatty liver conditions in humans. The rat liver attenuatioin coefficient slopes were stdied as a function of time postinjection of $CCl_4$ and the changes in measured ultrasonic characteristics were compared to pathological changes.

## THEORY

### A. Interaction of tissue and ultrasound

A brief review of basic phenomena on the tissue-ultrasound interaction is given here to assist in interpretation of our results. Ultrasonic attenuation in normal soft tissues is composed of absorption and scattering, and is known to increase approximately linearly with frequency over the 1-10 MHz range commonly used in ultrasonic diagnosis.

The primary mechanism for the absorption of sound in biological material is the macromolecular relaxation process over a broad range of frequencies (Parker *et al.* 1984).

Mathematical models of absorption exist to generate the linear-with-frequency increase in attenuation or to produce an absorption curve with properly shaped distribution functions of relaxation mechanisms over a finite band of frequencies.

However, the values of absorption measured within 1-10 MHz are insufficient for meaningful interpretation of the underlying relaxation process.

Another important ultransonic property is the scattering of sound waves as a function of angle and frequency. The pattern of scattered pressure is shown to have a Fourier transform relation to the structure of inhomogeneities within a sample volume of the tissue interrogated by an ultrasound beam.

For the liver, which is regarded as a random medium, average scattered intensity has a Fourier transform relationship to the correlation function of inhomogeneities in the medium.

If scattering were produced by small sized, randomly distributed scatterers, it would be omnidirectional and would increase with frequency to the fourth power.

As the total power scattered per unit volume by liver is not an appreciable fraction of the incident power in the low frequency range, scattering is a minor contributor to total attenuation and therefore absorption processes dominate.

### B. Effects of carbon tetrachloride

Carbon tetrachloride toxicity has been carried on for the study of drug sensitivity and the biochemical mechanisms have been extensively documented. Some major effects are summarized below.

For mature rats and other mammals, the primary toxic effects of $CCl_4$ are localized to the liver as metabolism produces cleavage of the $CCl_3$-Cl bond, which in turn results in major pathological damage to the endoplasmic reticulum of the cells. Here, lipid peroxidation is thought to be the most important immediate consequence of the lethal cleavage.

These events are the beginning of a complex mechanism of biochemical and structural changes. At doses around 0.25 ml $CCl_4$/100g body weight in adult rats, the toxicity results in a fatty liver within 24 hours.

At this time postinjection, the resulting necrosis and fat deposition are not uniformly distributed throughout all cells, but vary with position within liver lobules.

As a general statement, however, the fatty liver produced at 24 hours postinjection can be thought of as being swollen with increased fat and water content compared to normal livers.

### C. Spectral analysis of echo signal

The ultrasonic wave irradiated from the transducer inherently have a finite amplitude with specified frequency components, and undergo complex interaction along with the tissue that is being investigated. The resulting echo signals reveal fluctuations in both amplitude and frequency.

For imaging purposes, the ultrasonic system demodulates the returned echoes and detects the envelop signals, which in turn causes information losses to the ultrasonic image.

In attenuation work, the raw echo signals are acquired using the special hardware and analyzed in the frequency domain, which is called the spectral analysis.

A brief review of the mathematical model on the ultrasonic attenuation is given here.

For a section of soft tissue, having a thckness d, the transfer function in pulse-echo method, denoted by $|H(f)|$, can be expressed as[Kuc 1984, 1985).

$$|H(f)| = \exp\{-2\,a(f)\,d\} \qquad (1)$$

where f is the frequency in Hz and a(f) is the frequency dependent acoustic attenuation.

After the ultrasonic pulse propagates through the tissue, the power spectrum of echo signal Pe(f) has a relationship to that of incident pulse Pi(f) as follows:

$$Pe(f) = Pi(f)\,|H(f)| \qquad (2)$$

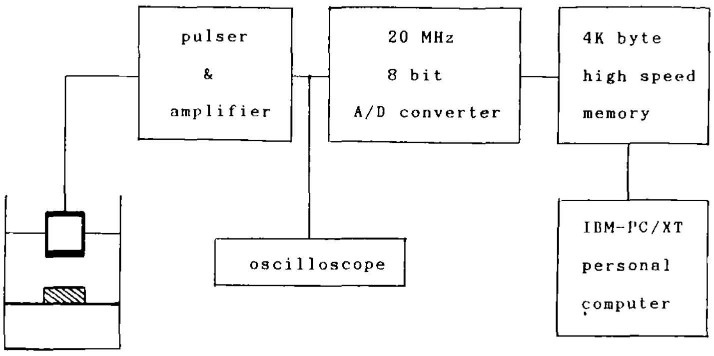


**Fig. 1.** Block diagram of the data acqusition and processing system.

Substituting eq.(1) into eq.(2), we have

$$Pe(f) = Pi(f) \exp\{-2\,a(f)\,d\} \tag{3}$$

To get the attenuation a(f) from eq.(3), taking the natural logarithm to both sides results in

$$a(f) = \frac{\ln Pi(f) - \ln Pe(f)}{2d} \tag{4}$$

Considering the linear-with-frequency increase in attenuation process, $a(f) = ao\ f$, we get the attenuation coefficient slope as follows:

$$ao = \frac{\text{slope}[\ln Pi(f) - \ln Pe(f)]}{2d} \tag{5}$$

## MATERIALS AND METHODS

Adult Wistar rats, weighing approximately 250 gr, were used in this study. Rats were injected with a dose of 0.25 ml CC14/100 gr body weight, administered intaperitoneally. Animals were killed and livers were excised at 5h and 24h postinjection.

The number of rats involved were six in each group. An additional group of three received no injections and were used as controls.

For the ultrasonic measurement of attenuation, whole livers were excised and immedicately placed in physiological saline. Measurements were made at room temperature within 2 hours of excision.

Sections of liver in each case were taken and kept frozen for pathological inspection of fatty changes. Lobes of the excised livers were positioned on a flat stainless steel reflector and placed in a water bath with a 2.25 MHz unfocused, wideband ceramic transducer mounted 7 cm above.

For each liver sample, 10 independent ultrasonic scans were performed and the echo signals were digitized with 20 MHz sampling rate and 8 bit resolution, which are fed to IBM-PC/XT digital computer for attenuation calculation.

A block diagram of the data acquisition system is shown in Fig. 1. In spectral analysis, the digitized

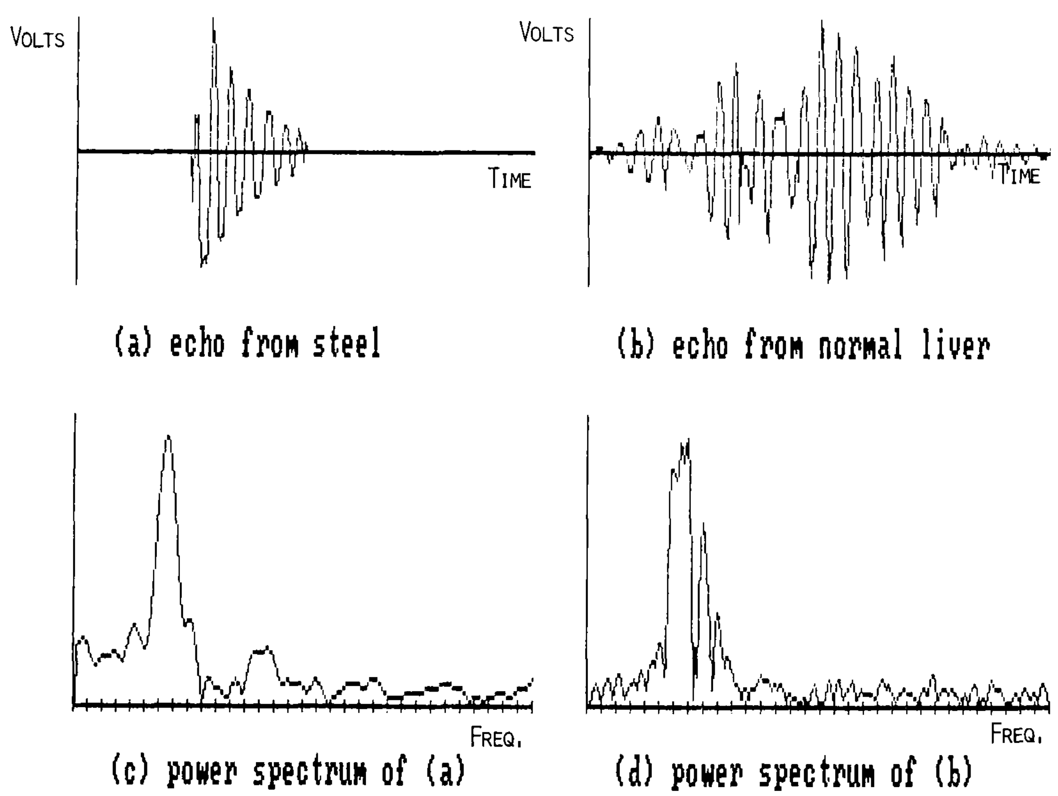


**Fig. 2.** Echo signals and corresponding power spectral densities for reference stainless steel reflector and normal liver.

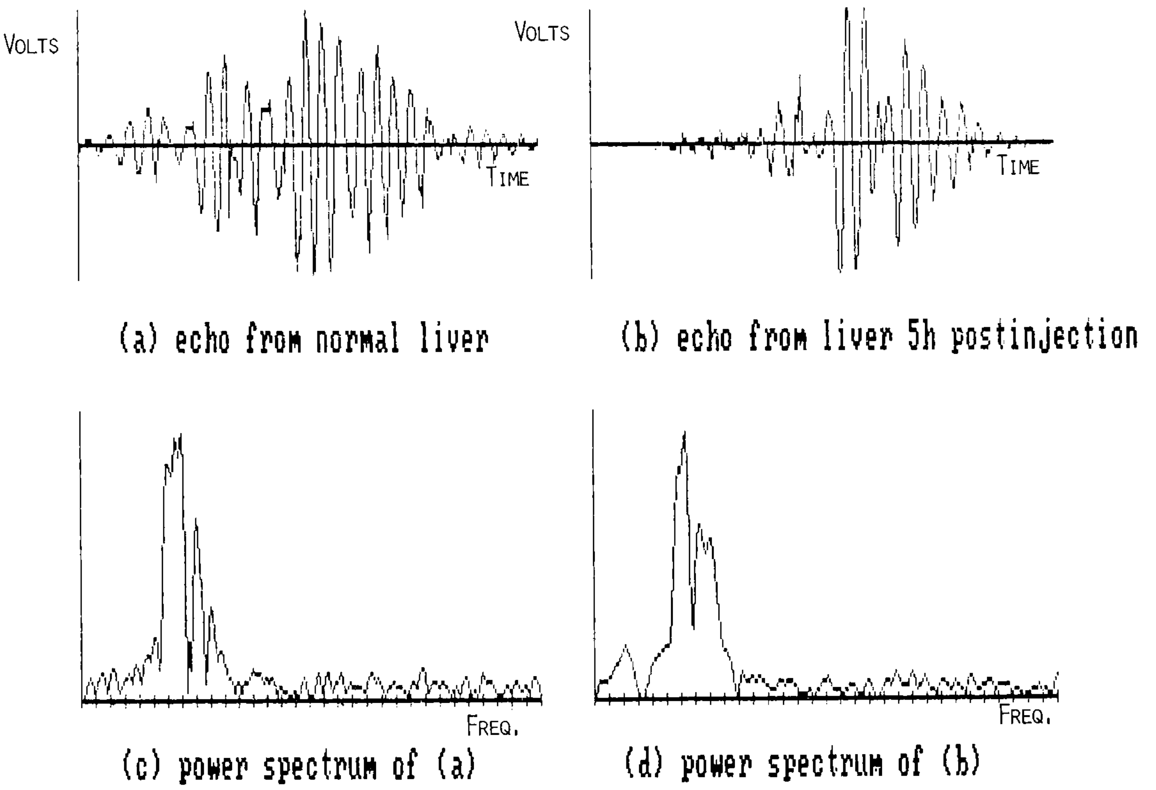


**Fig. 3.** Echo signals and corresponding power spectral densities for the normal liver and 5h postinjection. Slight deformation of power spectral density over the low frequency range is observed in (d) compared to (c).

echo signals were Fourier transformed and ensemble-averaged for each scan line of the liver. Attenuation coefficient slopes were calculated from the resulting power spectral densities using the least square fitting method.

The estimated attenuation values were compared to the pathological investigation.

## RESULTS

The echo signals and their corresponding power spectral densities are shown in Figs 2, 3, and 4. As described in the text, the power spectral density of echos from the reference stainless steel reflector revealed smooth shape near the center frequency (peak), while those from the liver contained fluctuations due to the irregular and inhomogeneous structure inside the liver tissue.

The estimated attenuation coefficient slopes are summarized in Table 1 and Fig. 5.

Attenuation values were found to vary with time, showing the decreasing attenuation coefficient slopes as the amount of fat change increased.

For the control group, the mean value of three was obtained 0.64 dB/Mhz.cm and the others 0.54 dB/MHz.cm, 0.49 dB/MHz.cm for 5h and 24h postinjection respectively. Although overlaps exist among the measured attenuation values of adjacent group, the mean slopes reflect the relative amount of fat in the liver that could be a useful diagnostic parameter.

## DISCUSSION

In this experimental study of ultrasonic characteristics, the attenuation coefficient slopes were investigated using digital spectral analysis of the ultrasonic echo signals. There appeared to be no simple relationship between the component of the many biochemical and structural changes in the rat liver affected by $CCl_4$ and the corresponding changes in ultransonic attenuation.

Since fat is often reported to have lower attenuation than normal liver (Parker 1986), the increase in fat content, coupled with cellular and whole organ swelling, would act to lower the measured attenuation slope as a function of time postinjection.

Also the increase in the size and number of fat droplets, having different density and compressibility from the surrounding intra-and extracellular components, and the structural cleavage of liver lobules might be expected to change the ultrasonic scattering process. This may lead to the variations

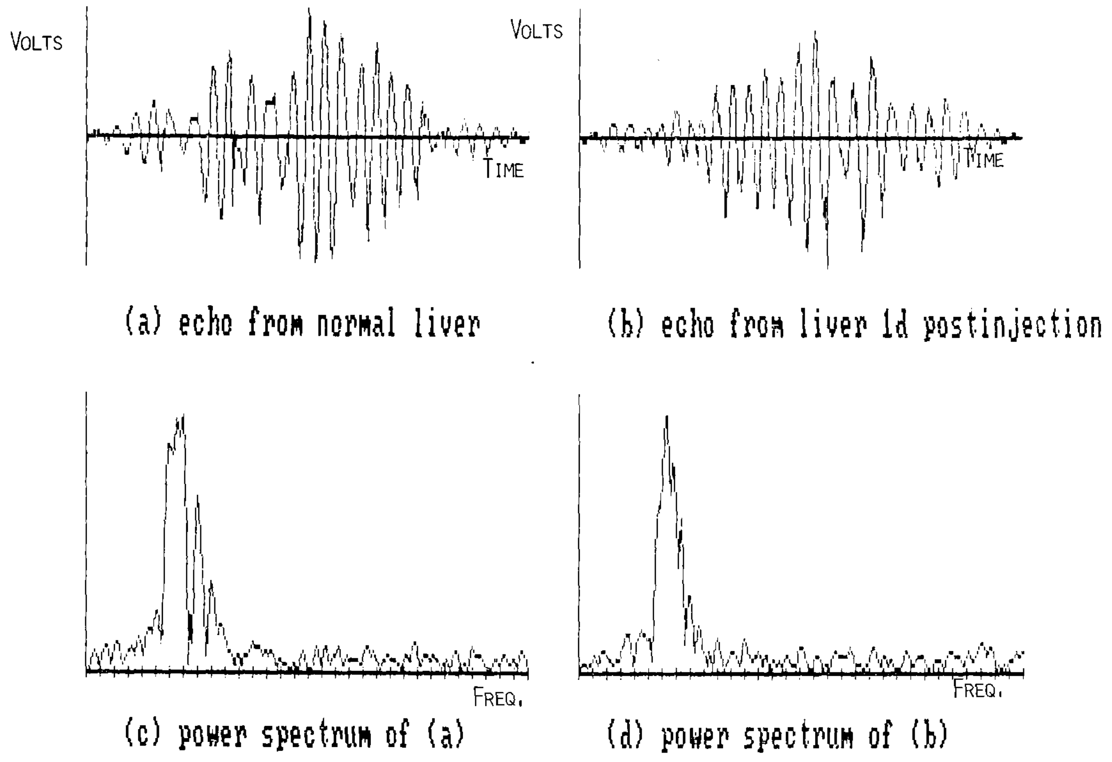


**Fig. 4.** Same as in Fig. 3 except for 24h(1d) postinjection. The power spectral density in (d) shows relatively small fluctuation, leading to the good estimation of attenuation coefficient slope.

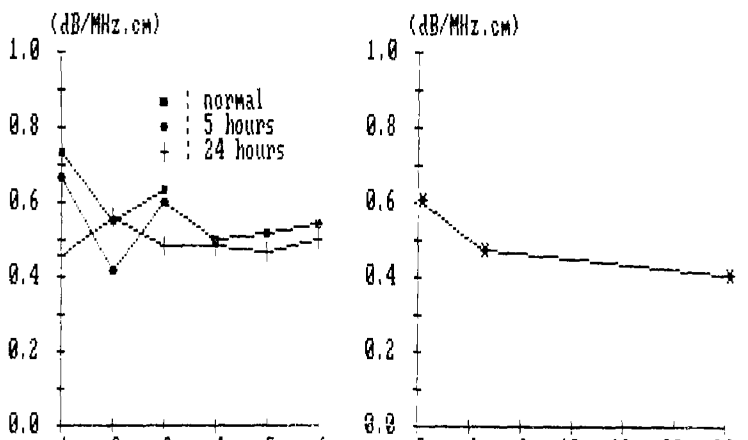


**Fig. 5.** Plot of estimated attenuation coefficient slope (left). The mean values are also shown (right) for three groups along with the time postinjection.

and overlaps of the measured ultransonic attenuation values.

As mathematical model used in this study assumes the linear-with-frequency increase in attenuation, suitable homogeneity and no focal deformation are required for the correct estimation of attenuation coefficient slopes.

These results are relevant to clinical studies of attenuation in-vivo. First, the data presented herein show that attenuation coefficient slopes can vary with time due to the increase in fat content in the liver. Thus, a characterization of this form of fatty liver could be given by a simple lower-than-normal generalization with regard to the staging of the disease or time dependence of the measurements. This may prove to be the case in forms of cirrhosis in humans.

Second, the attenuation results show that both the magnitude and frequency dependence of attenuation are important parameters. Some measurements of attenuation are in fact estimates of an attenuation coefficient with different center frequencies of transducers (Parker 1984) which is related to true attenuation in cases where the liner-with-frequency conditions do not hold. However, abnormal livers of comparably low and diffuse fatty changes are expected to have frequency characteristics with finite bandwidth and slight deformation in shape over the low MHz frequency range. Thus attenuation coefficient slopes based on the linear-with-frequency model could be applicable tothe measurement of frequency dependent attenuation with reasonably wideband ultrasonic transducers and signal processing hardware.

Some of the problems in research for the correct estimation of attenuation in the soft tissue are the

**Table 1.** Comparisons of the estimated attenuation coefficient slopes and corresponding pathological changes

| Group | Case | ao(dB/MHz.cm) | S.D. | Pathological change | |
|---|---|---|---|---|---|
| *control* | 1 | 0.73 | 0.07 | | |
| | 2 | 0.55 | 0.11 | normal | |
| | 3 | 0.63 | 0.06 | | |
| 5h | 1 | 0.67 | 0.11 | mild and diffues microvesicular | * |
| | 2 | 0.42 | 0.06 | intracytoplasmic fatty change | |
| | 3 | 0.60 | 0.12 | 1: sinusoid foamy cell | - |
| | 4 | 0.50 | 0.15 | 3: no change | |
| | 5 | 0.52 | 0.10 | | |
| | 6 | 0.54 | 0.02 | | |
| 24h | 1 | 0.46 | 0.06 | foamy cell change | |
| | 2 | 0.56 | 0.04 | moderate fatty change | * |
| | 3 | 0.50 | 0.03 | | |
| | 4 | 0.48 | 0.06 | | |
| | 5 | 0.48 | 0.11 | | |
| | 6 | 0.47 | 0.10 | | |

ao: estimated attenuation coefficient slope
S.D.: standard deviation
*: no correspondence
-: no fatty change in both investigations.

elimination of unexpected specular echoes leading to significant error in attenuation estimates and the optimization of the linear fitting algorithm. Eventhough, in our *in vitro* study, it was possible to use the fixed experimental setup to get the coherent ultrasonic echo signals, slight modification in both the data acquisition system and signal processing algorithms is to be done from the viewpoint of clinical applications.

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=국문초록=

## 사염화탄소에 의한 실험적 지방 간 조직의 초음파 특성

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곽철은 · 민병구 · 최병인* · 유은실** · 김용일**

본 연구에서는 실험적 지방 간 조직의 초음파적 특성을 관찰하기 위하여 펄스-에코우 방법에 의한 초음파 감쇠 계수를 측정하였다.

지방 간 조직의 유발을 위하여 성인 쥐에 0.25 ml/100 gr의 사염화 탄소를 주입하고 5시간과 24시간이 경과한 후 간을 추출하여 초음파 반사신호를 얻었다. 15 예의 정상 및 지방 간 조직에 대한 초음파 스펙트럼 분석 결과 다음과 같은 결론을 확인하였다.

1. 사염화 탄소 주입 후 경과 시간에 따라 간에 축적된 지방의 양이 증가함을 관찰하였고, 이에 비례하여 초음파 감쇠계수가 감소함을 보였다.

2. 초음파 스펙트럼 분석 방법에 의하여 추정된 감쇠 계수는 범용의 초음파 영상 기기에서 손실되는 조직 특성화 정보로 사용될 수 있다.

### LENGENDS FOR FIGURES

**Fig. 1.** Abdominal radiograph of Wistar rat after the injection of carbon tetrachloride with the opaque dye.

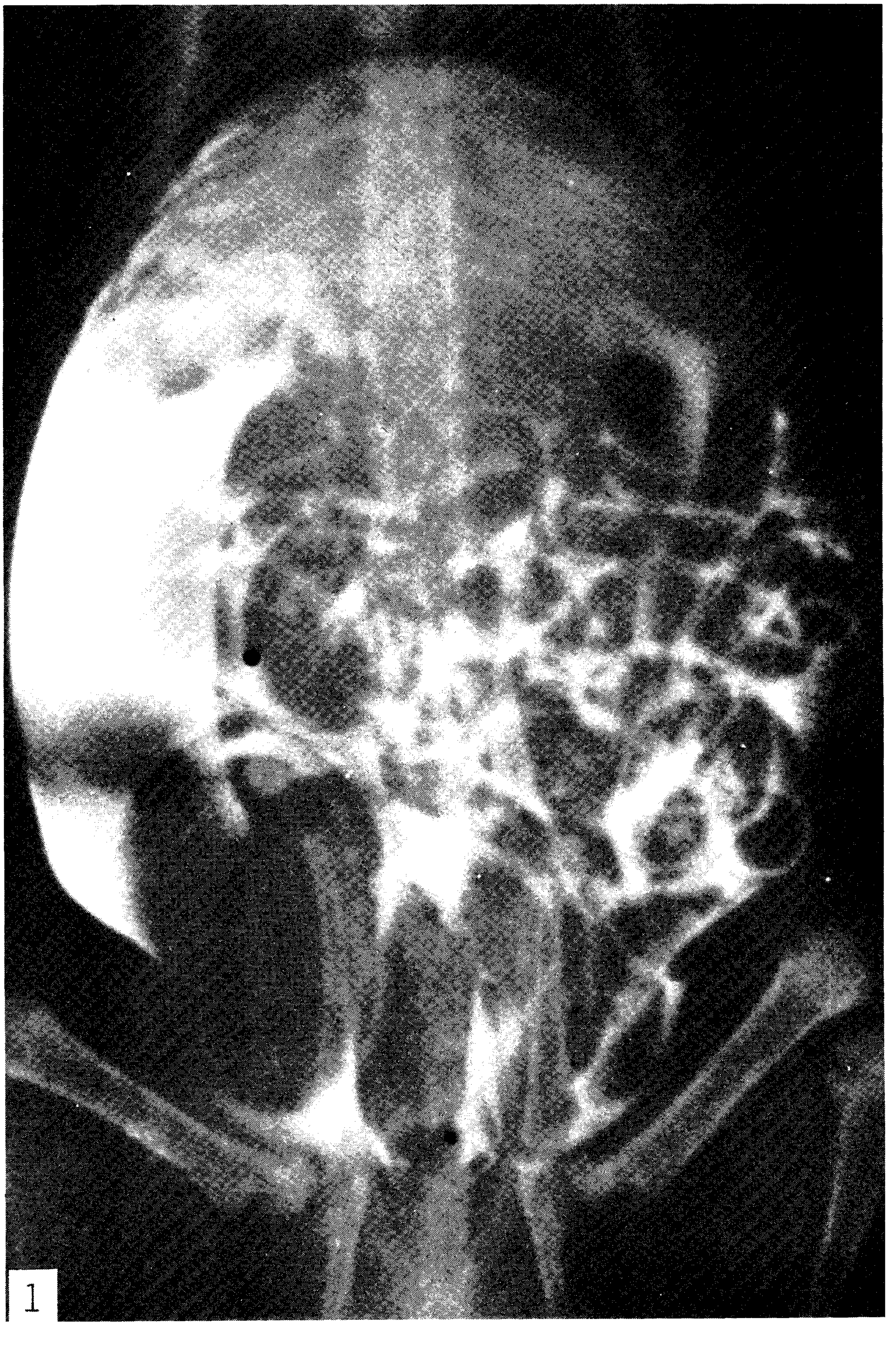
**Fig. 2.** Excised rat liver at 5h postinjection, with a dose of 0.25 mg/100 gr body weight. Thickness of the main lobe is approximately 1 cm.

**Fig. 3.** Excised rat liver at 24h postinjection.

**Fig. 4.** Micrograph of normal rat liver section, H & E x40.

**Fig. 5.** Micrograph of rat liver at 5h postinjection, H & E x40.

**Fig. 6.** Micrography of rat liver at 24h postinjection. Fat droplets appear to increase in size and concentration compared to normal and 5h postinjection, H & E x40.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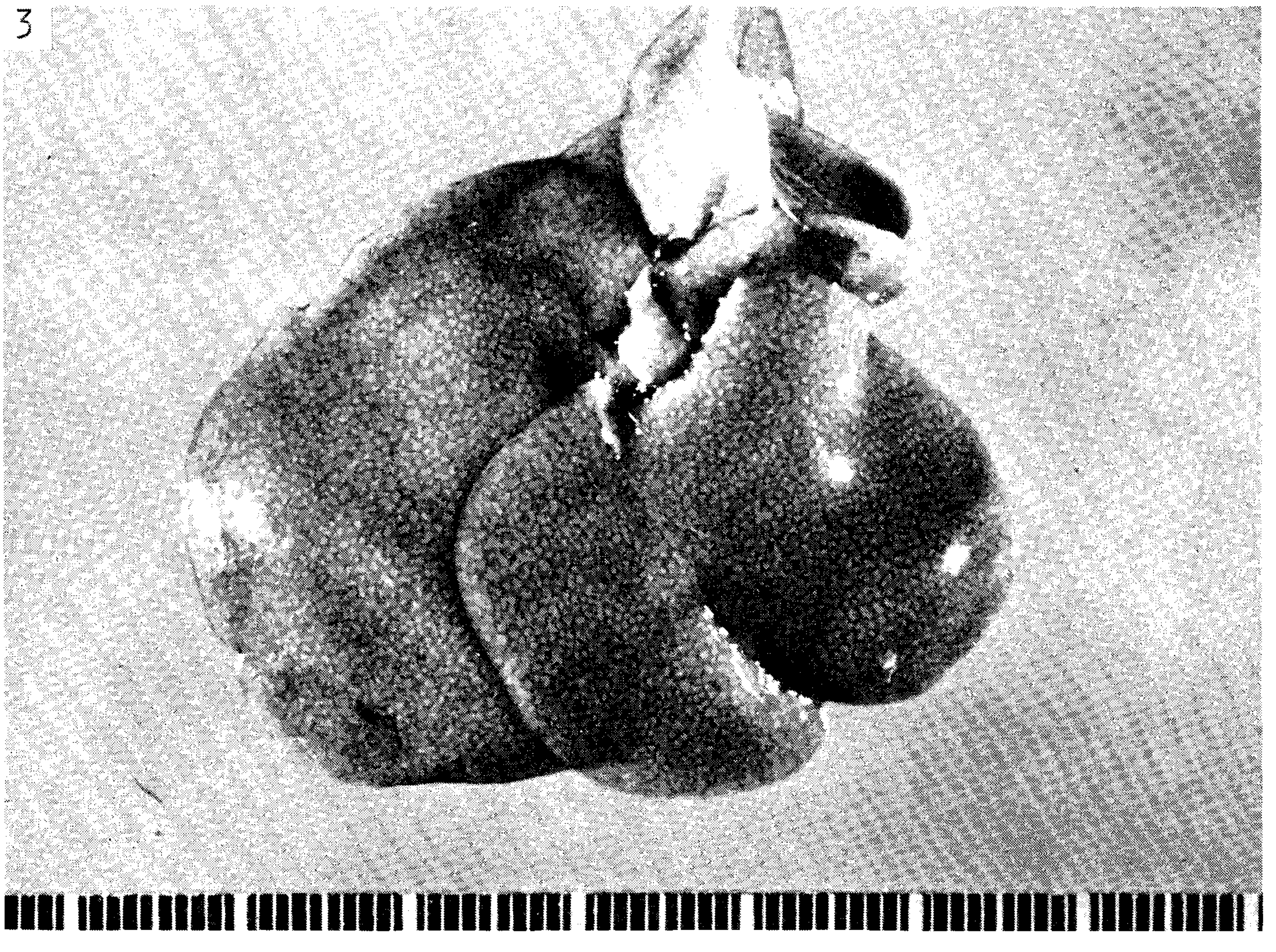

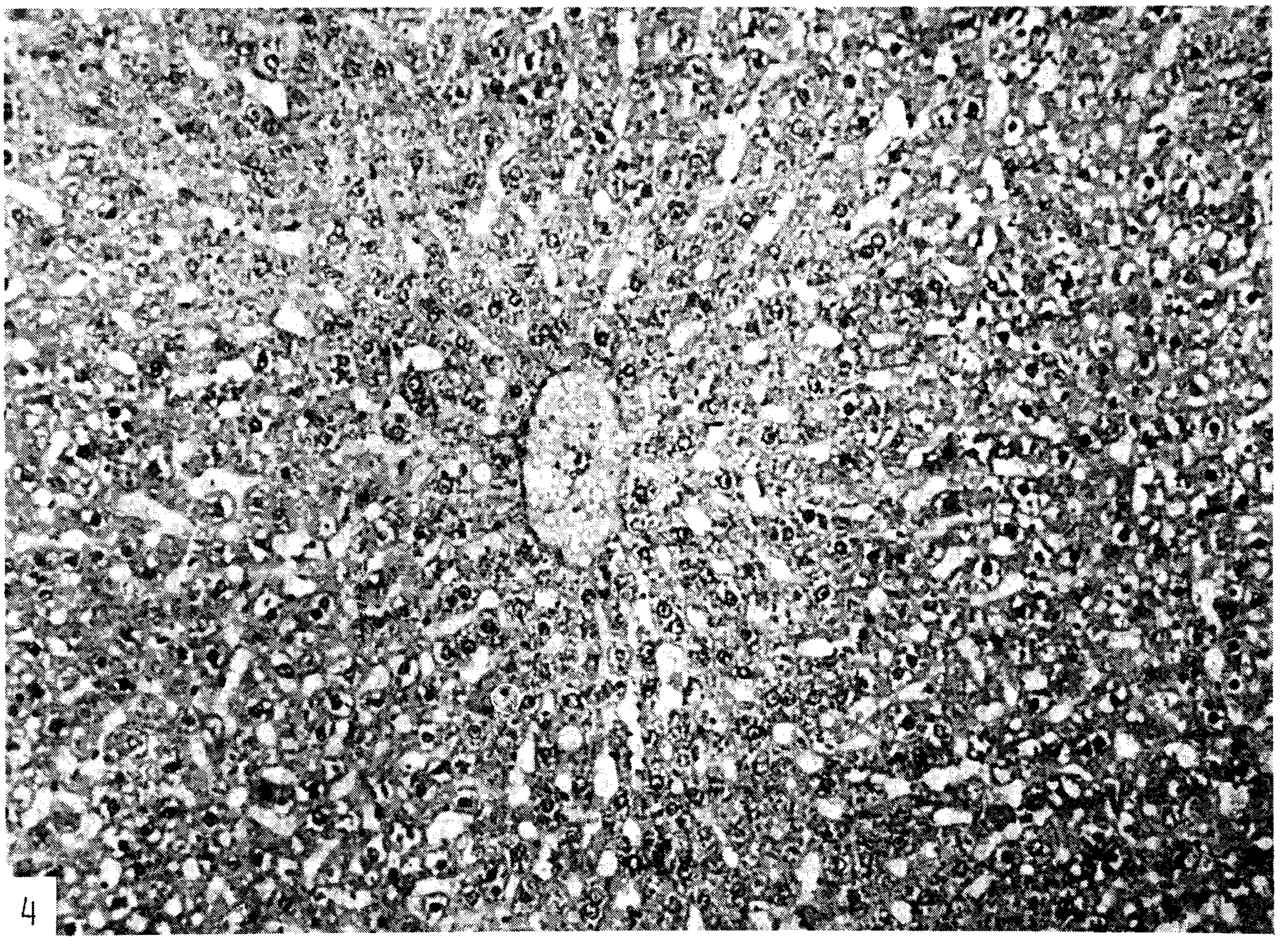

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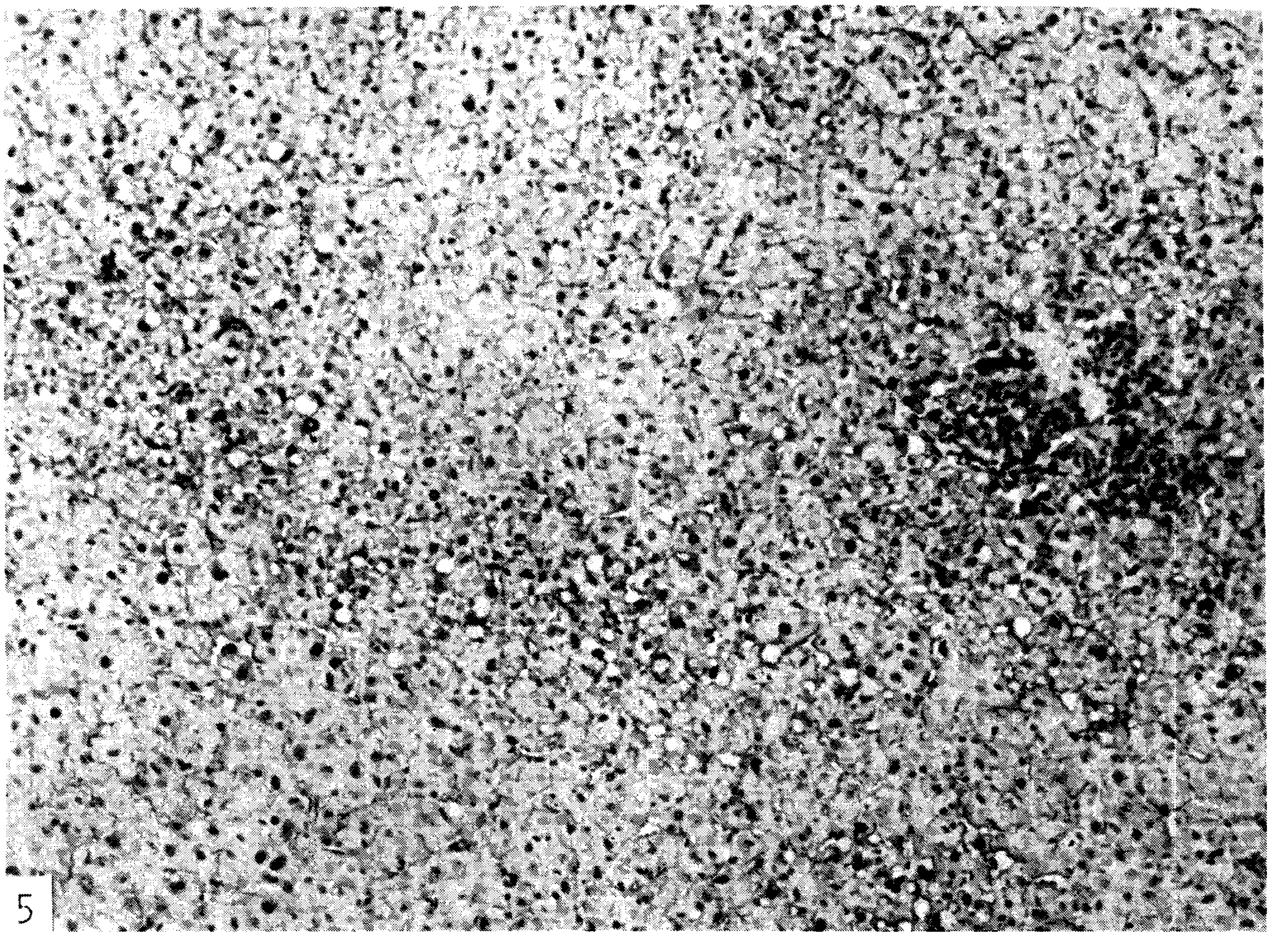

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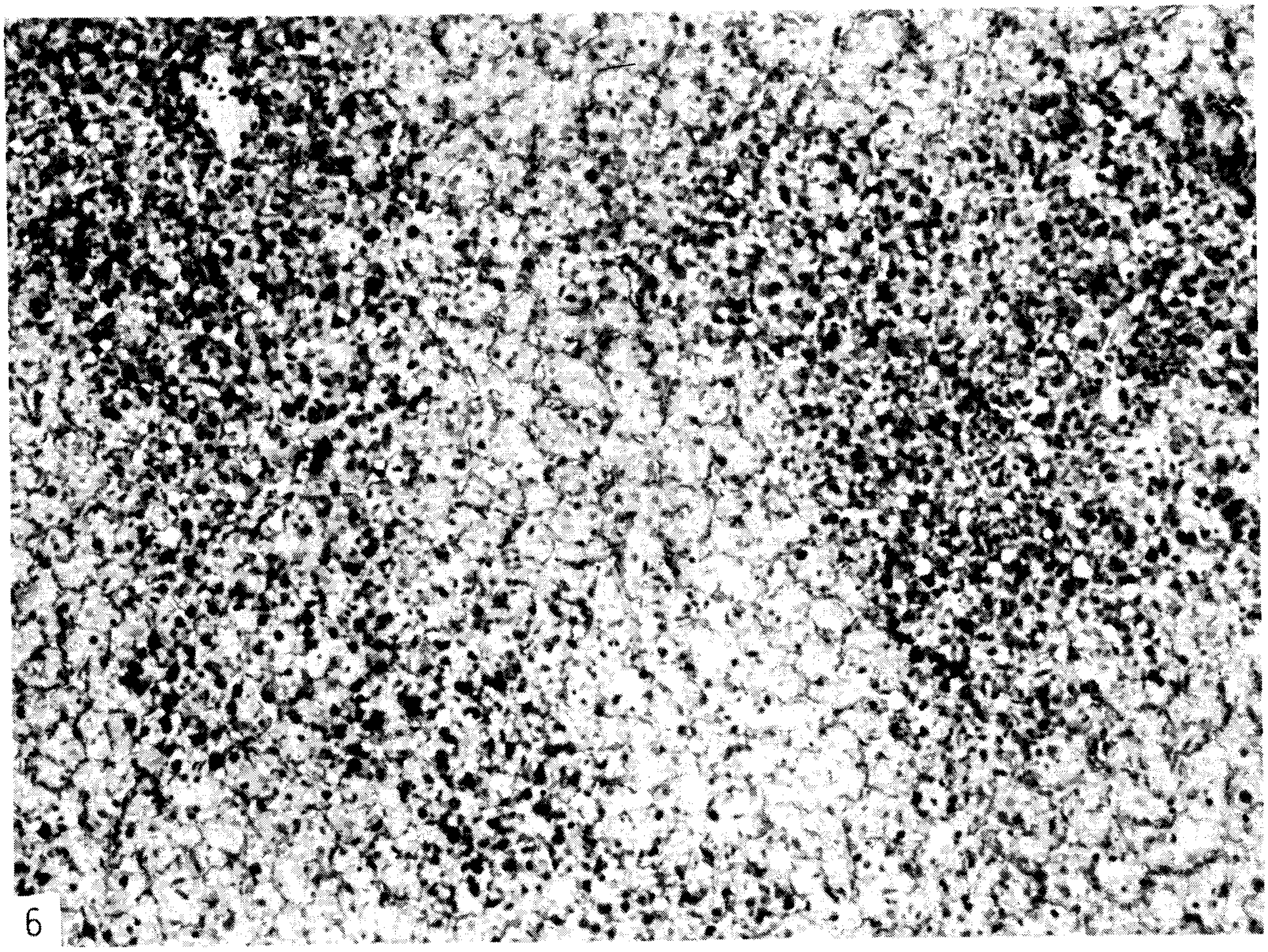

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