investigated in the dog the plasma levels of active renin, and of cryo- and acid-activated renin, before and after bilateral nephrectomy.

Material and methods. 3 male mongrel dogs with an average weight of 24.3±2.6 (SEM) kg were anaesthetized with 30 mg/kg sodium pentobarbitone i.v. A catheter was introduced into the femoral artery for blood sampling. The kidneys were exposed extraperitoneally by a flank incision, and dissected free from the surrounding tissues. After a recovery period of 60 min renal arteries and veins were clamped simultaneously on both sides, and the kidneys removed within 1 min. Arterial blood for renin estimation was withdrawn 5 min before and at 0, 10, 20, 30, 60, 90, 120, 150, 180, 210 and 240 min after removal of kidneys. Blood was collected into ice-cooled tubes containing EDTA, and o-phenanthroline. Plasma samples were stored, at −20 °C, until assay.

The plasma concentration of total, active and inactive renin was determined, as described previously[7]. For the estimation of total renin (TPRC) and active renin concentration (APRC) plasma was dialyzed against 0.05 M-glycine buffer (pH 3.3 and pH 4.5, respectively) containing 5 mM EDTA and 90 mM NaCl. This was followed by inactivation of the angiotensinases by heating at 32 °C for 1 h. The plasma was then incubated with excess sheep renin substrate at pH 7.4 for 1 h, as described by Skinner[4] and angiotensin I measured by radioimmunoassay (Lijnen et al.[8]). The difference between TPRC and APRC is described as the inactive plasma renin concentration (IPRC).

The cryoactivation of renin[6] was investigated by measuring the plasma reinin activity in plasma samples immediately after thawing and after storage of the plasma at −5 °C and at pH 7.4 for 4 days. The value of PRA before and after the exposure of the plasma to the cold is called respectively active (APRA) and total plasma renin activity (TPRA). The calculated difference between TPRA and APRA is considered as inactive plasma renin activity (IPRA). Plasma renin activity was measured by radioimmunoassay of the angiotensin I which was generated during incubation of the plasma samples for 1 h at 37 °C with the endogenous renin substrate at pH 6.0[9] according to the method of Fyhrquist and Puutula[10].

Results and discussion. As shown in the figure, the control values (average of time zero and 5 min before) were 3.5±0.65 (SEM) ng/ml/h for TPRC and 3.43±1.51 ng/ml/h for APRC. The concentration of acid-activated inactive renin was very low (0.07±1.14 ng/ml/h). In the control period TPRA averaged 1.25±0.26 ng/ml/h, and APRA 1.95±0.45 ng/ml/h. Cryoactivated inactive renin could not be demonstrated. Likewise, after nephrectomy IPRC and IPRA could not be demonstrated by acid activation or cryotreatment respectively. This is in contrast to the findings in human plasma where renin can be activated by various in vitro procedures such as acidification, exposure to cold or treatment with proteolytic enzymes[5]. Cryo- or acid activation of inactive renin has also been demonstrated in the plasma of several other species such as pigs[11], rabbits[12] and rats[13].

After bilateral nephrectomy the clearance of total and active renin from dog plasma is similar (fig.). Acidification and cryotreatment of dog plasma do not seem to interfere with the disappearance of renin.

On average, plasma renin (TPRC, APRC, TPRA, APRA) fell to half its initial value within 30 min; this is in agreement with previous studies[3,14].

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PRO EXPERIMENTIS

Demonstration of particulate accumulations in solid tissue[1,2]

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Summary. We describe a histological clearing procedure involving partial tissue hydrolysis by pancreatin and demonstrate its application. Changes in distribution of 5.7-μm latex particles in mouse liver and heart muscle 6 months after i.v. latex injection were quantitated. The method is useful for studies of long-term redistribution of particles in vivo and for locating large, infrequent particles which are difficult to find by ordinary tissue-sectioning techniques.

Many satisfactory procedures are available for studying the disappearance of small, nondegradable particulates from blood and their accumulation in the tissues of the reticuloendothelial (RE) system[4]. Methods for following the subsequent fate of such particles, however, are not as well developed. The present report describes a clearing procedure which is useful for studies of the storage and redistribution of particles within tissues. The procedure is a modification of a KOH-glycerol clearing process previously applied to mouse Peyer's patches and mesenteric lymph nodes for the demonstration of accumulated 2-μm latex particles[5]. The earlier technique gave satisfactory results when applied to pale tissues, but was less successful with tissues such as liver and spleen. The new procedure, which utilizes partial tissue hydrolysis by pancreatic enzymes, successfully clears all types of soft tissue and is well suited

to studies of small-scale rearrangements of particles within tissues.

Materials and methods. 10-week-old female Swiss mice (Hale-Stoner strain) were injected in the tail vein with 0.05 ml saline containing 1.15×10^8 styrene divinyl benzene latex particles (Dow Chemical Co.) of mean diameter ± SD 5.7 ± 1.5 µm. The sterile latex suspension was sonicated in a water bath sonicator for 30 min before injection to disperse aggregates. Microscopic observation and Coulter sizing indicated that the number of aggregated particles was negligible. Groups of 4 mice were killed by ether overdose 1 day and 6 months after injection and viscera were fixed in acetate-buffered formalin. After 1 week of fixation, 0.5-mm tissue slices were cut with a razor blade. Central cross-sections were taken from heart; liver slices were taken from the region adjacent to the gallbladder. Similar samples were taken from non-injected control mice.

The clearing procedure was adapted from the pancreatin method of Dingerkus and Uhler[6] who applied it to gross specimens for the determination of skeletal structure. Porcine pancreatin (CalBiochem Co., 4×) was made up to 1% in sodium borate buffer prepared by adding 30 parts saturated sodium borate solution to 70 parts distilled water. The crude pancreatin solution was passed through a series of Millipore filters of decreasing pore size, the last being 0.2 µm. The filtered pancreatin solution was stable under refrigeration and could be used for at least 6 months. Slices of formalin-fixed tissue were well rinsed in water and placed in a solution of 80 parts 95% ethanol and 20 parts glacial acetic acid for 1 day. The slices were hydrated through a series of decreasing ethanol concentrations (95%, 70%, 40%, 15%) to water. The tissue slices were then placed in the pancreatin solution for 1–3 days at room temperature. Enzyme treatment was terminated when the specimens were partially bleached and translucent. Incubation at 37 °C greatly accelerated enzyme action, but was not used because the tissues tended to become overhydrolyzed and fragile. After pancreatin treatment, the tissue slices were placed in 0.5% KOH for 12–24 h and then transferred through a series of 0.5% KOH-glycerol solutions (3:1, 1:1, 1:3) for a day each. The cleared slices were preserved in glycerol containing a crystal of thymol. For observation, tissue slices were placed in a depression slide in a drop of glycerol.

Results. Latex-injected animals suffered no apparent ill effects during the 6 months of the study. Spleen, liver, and lungs were grossly normal in animals sacrificed at both 1 day and 6 months.

Despite the transparency of cleared tissue, major structures such as blood vessels could be distinguished. Latex particles were readily discerned by their roundness and refractility. No particles resembling 5.7-µm latex were seen in tissues of non-injected control mice.

6 months after injection, the overall abundance of particles was increased in liver but decreased in heart muscle. The most striking change was the formation of numerous aggregates in liver. Latex was widely dispersed as single particles 1 day after injection (fig. 1). The only aggregates were a few

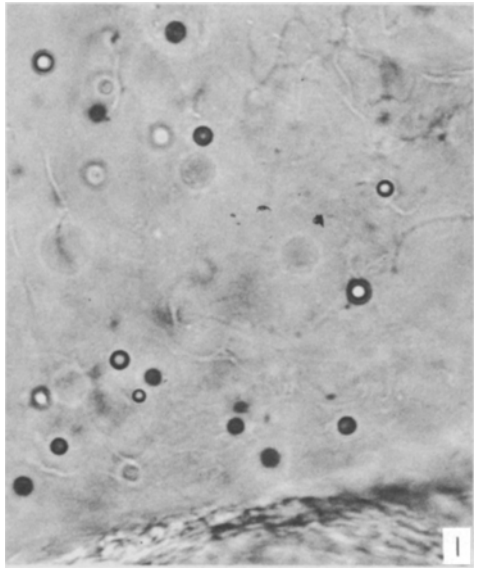

Fig. 1. Cleared liver tissue 1 day after i.v. injection of latex. Particles are distributed singly. Particles above plane of focus appear dark. Many fine connective-tissue fibrils are visible. Portion of a portal vein appears below. ×375.

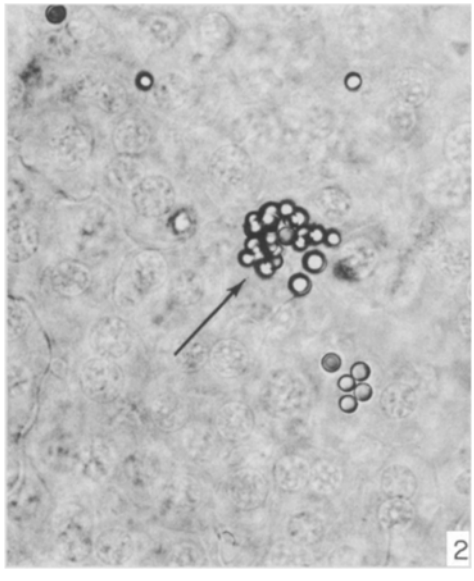

Fig. 2. Cleared liver tissue 6 month after i.v. injection of latex. A few particles are distributed singly, but most are clustered into an aggregate containing approximately 20 particles (arrow). Nuclei of hepatic parenchymal cells are faintly visible. ×375.

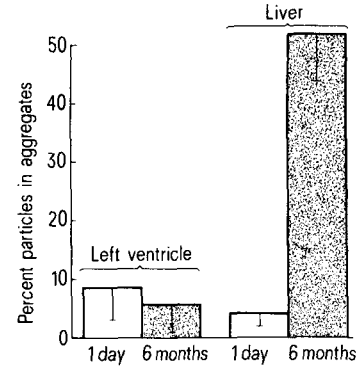

Fig. 3. Latex particle distribution in liver and heart (wall of left ventricle) 1 day and 6 months after injection. Left bar of each pair shows percent of latex particles present as aggregates at 1 day; right bar shows percent present as aggregates at 6 months. Vertical lines give SD. Each bar shows results of classification of 1000 particles from each of 4 mice.

doublets. At 6 months, both single particles and aggregates were present (fig. 2). Most aggregates consisted of tight clusters of 8 to 15 particles, but a few contained as many as 30 particles.

Figure 3 quantitates the difference between liver and heart muscle with regard to aggregate formation. 1000 consecutive particles were surveyed at magnification $\times 100$ in cleared tissue from each mouse of the 1-day and 6-month groups. As each particle was counted, it was classified as single or component of an aggregate (2 or more particles in apparent contact). The bar graph shows that less than 10% of particles in both liver and heart were aggregated at 1 day. At 6 months the percent of aggregated particles in heart was not significantly changed, but $51.9 \pm 8.0\%$ of the particles in liver were grouped in clusters of 2–30 particles ($p < 0.001$, liver, 1 day, vs liver, 6 months).

Discussion. The clearing procedure described here is a useful contribution to the investigation of particle retention in tissues. Small numbers of single particles or infrequent clusters can be discerned within thick slices of tissue made transparent by the clearing process. An incidental, but sometimes valuable, attribute of the method is that it avoids the use of xylene-based solvents which dissolve many types of polymer particulates used in studies of RE function.

The present study involved determination of the presence of 5.7-μm latex particles in mice 1 day and 6 months after their injection into the tail vein. Large numbers of particles were found in tissues, e.g., heart muscle, in which tissue macrophages do not ordinarily make contact with circulating blood. Although the mean diameter of the particles was considerably less than the probable diameter of most limiting vascular passages[7], some particles were obviously trapped within capillaries in the heart. Interactions between endothelial and particle surfaces and 'stickiness'-promoting substances such as fibrin determine the arrest of particles which are nominally small enough to pass through capillaries[8]. The precise mechanism or mechanisms of trapping cannot be determined from the present study.

In 6 months no overt rearrangement of particles occurred in heart muscle, a tissue known to have little RE function (fig. 3). In contrast, marked aggregation took place in liver, a major component of the RE system. Progressive aggregation of nondegradable particles phagocytized by hepatic macrophages has been described previously[9,10], but rarely with such large particles or in quantitatable form. We postulate that the mechanism of this aggregation involves repeated macrophage ingestion of other, possibly dead or dying, particle-containing macrophages. Although it canot provide definitive answers to questions of mechanism, the method described here permits determination of the fate of particles within tissues over a wide range of particle concentration and can be usefully applied to a variety of problems in histopathological research.

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2 The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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