Distribution of Carbon after Intravenous Injection in the Normal Rabbit. Leakage into the Synovium but not Other Non-Reticuloendothelial Tissues

Carbon black (India ink) is widely used as a tracer to mark sites of vascular injury[1]. In a recent study of monkey synovium, carbon repeatedly leaked from normal synovial venules[2]. Since such leakage in normal animals with the doses used has previously been considered to occur only in reticuloendothelial tissues (liver, spleen, bone marrow)[1], I have compared the amount of carbon labelling in synovium and a variety of other tissues in another animal, the rabbit.

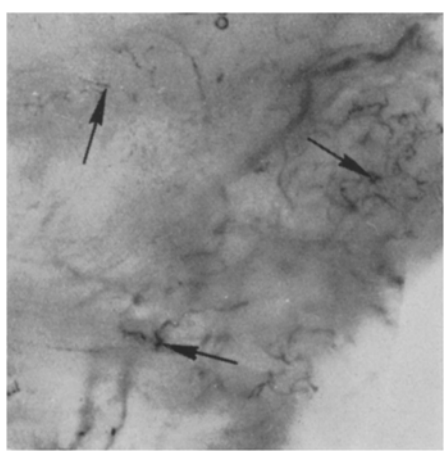
Seven healthy New Zealand white rabbits, weighing 1.8–2.4 kg, were used. 2 rabbits were lightly anesthetized with i.v. sodium pentobarbital (12–15 mg/lb). The others were injected while restrained. Filtered carbon black (Günther Wagner, Pelikan-Werke, Hannover, Germany) 0.1 cm^3/100 g was injected into an ear vein. Animals were sacrificed 1 and 2 h later by overdosage of pentobarbital. Knee synovium, skin over the abdominal wall, diaphragmatic and gastrocnemius muscle, peritoneal membrane, mesentery, spleen, liver, submaxillary glands, pericardium, kidney, lung, and rib cross section were promptly excised and placed in $^1/_2$ strength Karnovsky's paraformaldehyde-glutaraldehyde[3]. Portions were then divided for electron microscopy (EM), light microscopy, and clearing in glycerine for examination under the dissecting microscope. Tissues for electron microscopy were processed as previously described[2]. Quantitation was attempted by counting numbers of leaks per total capillaries and venules seen by electron microscopic and paraffin sections. By dissecting microscopy both this measure and number of leaking vessels per area were calculated.

No carbon was found by any technique in muscle, mesentery, submaxillary glands, pericardium, peritoneum. Massive carbon deposition was found by all techniques in the reticuloendothelial tissues (liver, spleen and bone marrow). Very rare leaks were seen in skin, kidney and lung. In the skin carbon was seen only by dissecting microscope (0.70 carbon leaks were seen per 10 cm^2). In the kidney 0.75 carbon leaks were found per 10 cm^2 of renal cortex by dissecting microscope. Only one questionable label was seen on paraffin section. In the lung there was anthracotic pigment that could not be differentiated from the carbon by light microscopy. A few collections of carbon were seen in pulmonary macrophages by EM. No EM evidence of carbon was seen in skin, muscle, submaxillary gland or kidney.

Synovial speciemens by dissecting microscope invariably showed more carbon labelled vessels (Figure). These were focal and varied from 1.0–7.0 per cm^2 with an average of 1.8. Microscopic examination of paraffin sections showed 5% of vessels to be labelled. The percentage was in the same range by EM. The EM studies confirmed that the carbon was in endothelium or outside this layer and not just plugging the lumens. The 2 h interval after carbon injection allowed carbon to be largely cleared from the circulation. Leaking vessels were almost all venules.

These studies show considerably more evidence of leakage of carbon from synovial vessels than from vessels of any other non-reticuloendothelial tissue studied. Although I have presented some quantitation the exact magnitude of leakage are difficult to ascertain as tissues differ in their degree of vascularity and the predominant type of vessels. Sampling error can also occur and because this would be greatest with EM I did not try to compare numbers of leaks at the EM level. Support for the concept of greater leakiness of synovial vessels comes from previous studies[4,5] that have suggested that bacteria and proteins pass more readily from the vascular system into joints than into several other body cavities (cerebrospinal fluid and ocular aqueous humor). The 250 Å carbon leaks largely from venules. It is from these vessels that carbon has been described to escape in chemically mediated vascular leakage while more seems to leak from capillaries after direct injury[6]. Phagocytosis of carbon by synovial venular endothelium is common[2] although rarely described in other normal non-reticuloendothelial tissues after similar doses of carbon[7]. Thus synovium handles this i.v. injected tracer somewhat as a reticuloendothelial tissue. The reason for this is not clear but for whatever reason this leakage into the synovium and thence into the joint space may be important in the localization to the joint of the yet unknown agent or agents causing rheumatoid and other inflammatory arthritis.

Résumé. Le charbon noir, administré par injection intraveineuse, s'échappe des vénules synoviales normales, mais pas des vénules de tissus non-réticuloendothéliaux. Cette conclusion vient à l'appui d'autres études qui révélèrent un plus haut degré de perméabilité des vaisseaux synoviaux, qualité peut-être importante pour la localisation, dans l'articulation, de l'agent (ou des agents) qui causent l'arthrite rhumatoïde ou d'autres arthrites inflammatoires.



Glycerine cleared rabbit synovial membrane. The dark marks (arrows) along the vessels indicate the many sites of carbon leakage into the vessel wall, found in this specimen.

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