Fate of particulate material arriving at the synovium *via* the circulation

An ultrastructural study

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Intravenously injected carbon has been shown to deposit in normal synovial venules in endothelial phagosomes and on both sides of the basement membrane (Schumacher, 1969). This study follows the subsequent handling of these particles after arrival at the joint *via* the circulation. Inert carbon particles have been proposed by Cochrane (1963) to localize in much the same way as other circulating macromolecules such as antigen-antibody complexes. Thus, study of the carbon distribution may provide clues to the synovial handling of these and other circulating particles that may be important in the pathogenesis of inflammatory joint disease. Intra-articular histamine was used to accentuate the carbon leakage in some animals. This technique allows comparison with a study by Cotran, La Gattuta, and Majno (1965) of carbon handling after histamine-induced leakage into muscle. Carbon in the synovium seems to escape more rapidly from the vicinity of the vessel.

Material and methods

21 2-kg. New Zealand white rabbits were used. Filtered carbon black (Gunther Wagner Pelikan-Werke, Hanover, Germany) was injected into an ear vein in a single dose (0.1 ml./100 g. body weight). Immediately after this 1 ml. histamine phosphate (1 mg./ml.) was injected into the right knee of eighteen animals. Opposite knees were not injected except in two instances in which equal volumes of normal saline were administered. Two rabbits each were killed at 5 min., 30 min., 90 min., 2 hrs, 3 hrs, 24 hrs, 48 hrs, 5 days, and 7 days after the injections. One rabbit each at 5 min., 30 min., and 48 hrs had only the carbon injections. Rabbits were killed with an overdose of sodium nembutal and the knee synovium promptly dissected while immersed in one-half strength Karnovsky (1965) fixative. Carbon labels were visible under the dissecting microscope and carbon-containing areas from both histamine-injected and non-injected knees were selected for further processing. Specimens were cut into 1 × 1 mm. portions and transferred to fresh fixative for 4 hrs at room temperature, washed in 0.1 M cacodylate buffer, post-fixed in osmium-veronal for 2 hrs, dehydrated with alcohol, and embedded

in Epon 812. Thick sections were cut with a glass knife on a LKB-2 ultramicrotome and examined for orientation. Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a RCA-EMU 3H electron microscope under a 50 KV beam.

Results

In all animals killed 5 minutes after the histamine and carbon injections much carbon was still present in the vascular lumina, some was phagocytosed by circulating platelets, and small amounts were already identifiable in endothelial vacuoles or lying between the endothelium and basement membrane (Fig. 1, opposite). Some intraluminal carbon seemed to be enfolded in endothelial processes. At these and subsequent examinations all vessels from which carbon had escaped were venules or capillaries, not arterioles. The manner of escape of the carbon from the synovial venules was identical with or without histamine but was best demonstrated after intra-articular histamine. Open gaps appeared at the junctions between venular endothelial cells. These were seen after from 3 to 30 min. and were identical to those described earlier in exercised joints (Schumacher, 1969) and in other tissues after histamine (Majno and Palade, 1961). Occasional leaks did occur in vessels with fenestrations but there was no evidence of passage of the carbon across endothelial fenestrations. Smaller particles, such as horse-radish peroxidase (50Å) and ferritin (100Å), appear to emerge from other capillaries across fenestrations (Clementi and Palade, 1969a).

At 30 min. only rare carbon still remained in the lumina and there were large deposits in endothelial vacuoles, extracellularly on both sides of the basement membrane, in pericytes, in intercellular tissue outside pericytes (Fig. 2), and in distant macrophages. Discontinuities in the basement membrane were frequent, but perfectly continuous venular basement membrane had also not been demonstrable in previous studies on other normal synovial vessels

FIG. 1 5 minutes after intravenous injection carbon is free in venule lumen, in vacuoles of a platelet or other cell process, and immediately outside the endothelium (at the arrow). $\times 30,000$

FIG. 2A 30 minutes after carbon injection the electron dense carbon particles are identifiable in endothelial vacuoles (V), between endothelium (E) and pericyte (P), and outside the pericyte basement membrane (arrow). The lucent area between endothelium and pericyte probably represents protein-poor oedema fluid. L = venule lumen. $\times 7,000$

FIG. 2B In addition to the carbon a PMN also lies outside the endothelium. $\times 5,000$

(Schumacher, 1969). In some areas the basement membrane appeared intact despite the escape of the carbon. Polymorphonuclear leucocytes (PMN) were associated with several areas of carbon leakage but were not seen at all sites. Pericytes were separated from endothelium by wide, clear areas of apparent oedema. Small numbers of carbon particles were seen in Type A synovial lining cells (Fig. 3) and in and around other synovial lining cells. Some vessels in this and all other specimens showed no leakage or other change from normal. At 90 and 120 min. the findings were still very similar to those at 30 min. Despite continued evidence of escape of some carbon to distant phagocytic cells, much still remained in the vessel wall in endothelium, pericytes, and intercellularly (Fig. 4). At 3 hrs there was less intercellular carbon but it was again easily identifiable in vascular endothelium, pericytes, macrophages, and synovial lining cells. Occasional PMN were still noted throughout the superficial synovium and these occasionally still contained some carbon in phagosomes.

By 24 and 48 hrs carbon was still seen in small deposits in the endothelium (Fig. 5). Carbon was now more prominent in vacuoles and dense bodies of pericytes, macrophages, and lining cells. PMN were no longer seen.

At 5 days intercellular carbon was very rare but deposits were still frequently identified in pericytes (Fig. 6), both Type A and B synovial lining cells, and macrophages or fibrocyte-like cells in the deeper synovium (Fig. 7). Much carbon was still present in phagosomes but in all cells some seemed to be free in the cytoplasm (Fig. 6). Adjacent to some typical phagosomal carbon deposits were very dense, fine granules not seen in normal animals and possibly representing a result of some carbon digestion or fractionation (Fig. 8). Some carbon-laden macrophages and lining cells were degenerating and had released carbon into the interstitium (Fig. 9).

At 7 days the findings were similar to those at 5 days. Carbon deposits were small and scattered. Very occasionally carbon was still seen intercellularly.

FIG. 3A Carbon particles (arrow) in vacuole of Type A synovial lining cell (A) 30 min. after injection. JS = joint space. B = Type B synovial lining cell. $\times 32,000$

FIG. 3B

FIG. 3B Process of synovial macrophage containing extravasated carbon 30 min after intravenous injection. L = lumen of superficial capillary. $\times 22,000$

FIG. 4 Large carbon collection between endothelium (E) and pericytes (P) 2 hrs after carbon injection. F = synovial fat cell. $\times 5,000$

FIG. 5 48 hrs after carbon administration small deposits are still visible in the endothelium (E) and in a pericyte. $\times 15,000$

FIG. 4

FIG. 5

FIG. 6 Carbon (arrow) in synovial pericyte 5 days after injection. The deposit is no longer surrounded by any identifiable phagosomal membrane. $\times 18,000$

At 5 and 7 days carbon was no longer identified in the vascular endothelium.

The largest carbon deposits were invariably seen in the histamine-injected knees but carbon was also seen at all times in the control knees.

Discussion

These studies have shown that portions of the intravenously-injected carbon tracer were retained in the vessel wall for as long as 7 days while other carbon escaped rapidly from the vicinity of the vessel and had reached synovial lining cells and presumably also the joint space as early as 30 min after injection. Thus these results suggest that both vessel wall and joint space are rapidly exposed to circulating particles and that either could be the prime site for the initiation of intra-articular disease. Other intravenously-injected materials of various sizes have also been shown to escape rapidly to the joint lining or joint space, but these have not been studied by electron microscopy. Such 'tracers' include bacteria (Shaffer, 1939), colloidal iron, trypan blue (Kling, 1938), and serum proteins (Bennett and Shaffer, 1939). These earlier studies could not examine the mechanism of leakage and, by focusing on the synovial fluid, did not show the sequence of handling of the particles within the synovial membrane.

The handling of carbon in the synovium is different from that reported by Cotran and others (1965) in

FIG. 7 5 days after carbon injection carbon is identified in a Type B synovial lining cell (B). L = vessel lumen. $\times 5,000$

rat muscle after local histamine application, predominantly in the rapid transfer of carbon toward synovial lining cells. Cotran and others (1965) did not note carbon in extravascular histocytes until after 4 hours and most carbon, even at 2 and 14 weeks, was seen in phagocytes lying along the vessel wall. The looser connective tissue of superficial synovium seems to be a likely explanation for this difference. Other findings in the two studies were quite similar. Cotran and others (1965) suggested that most carbon phagocytosed by endothelium was ingested from the basement membrane (rather than luminal) side after escape from the lumen through gaps between endothelial cells. Our results tend to support this, in that we found no clear examples of carbon being ingested from the lumen and there were many deposits in endothelium adjacent to gaps. Earlier studies emphasizing the initial stages of histamine-induced leaks in rat cremaster muscle by Majno and Palade (1961) and in mice by Alksne (1959) had shown carbon or HgS tracers largely blocked inside the basement membrane, but Majno and Palade (1961) did also note secondary phagocytosis by endothelial cells and transfer into perivascular histiocytes by 3 hours. Clementi and Palade (1969b), in studies of intestinal

FIG. 8 Synovial lining cells with very fine electron dense material adjacent to typical carbon particles (arrow). JS = joint space. $\times 33,000$

FIG. 9 Degenerating synovial lining cells apparently releasing some carbon into the extracellular space (arrow) 5 days after injection. $\times 8,000$

capillaries after EDTA and histamine perfusion, reported that most carbon was retained by the vascular basement membrane. Emigrating polymorphonuclear leucocytes (PMN) can be the mechanism for some increased spillage of carbon from the confines of the vessel basement membrane (Cotran and others, 1965; Hurley, 1964), but in the synovium some carbon quickly escaped from beneath the basement membrane before PMN had appeared and also escaped in animals not injected with histamine who had few PMN.

The carbon used in these experiments has generally been considered 'relatively inert' and has seemed to be well tolerated after phagocytosis. Whether the occasional deaths of carbon-laden cells and the apparent loss of phagosomal membranes are a result of the presence of the carbon is not known. Other non-living particles such as silica and urate crystals have recently been shown to break down phagosomal

membranes and to produce cell death (Weissmann, 1971; Schumacher and Phelps, 1971). The inert carbon in this and other experiments is used to show what may also be the localization of biologically important macromolecules. Although the latter would often be degraded by lysosomal enzymes, morphologically unidentifiable remnants may persist and may still serve as a source of antigen for immunological reactions or be otherwise toxic.

Summary

Intravenously-injected carbon escapes from normal synovial venules and to a greater degree from venules of rabbit knees after intra-articular histamine injection. Some carbon reaches the synovial lining cells and presumably the joint space by 30 min. Other carbon is retained in vessel walls (pericytes) for at least 7

days. Particulate materials arriving at the joint *via* the circulation might play a role in the pathogenesis of various types of joint disease. The demonstrated handling of the 300 Å carbon particles suggests that other similar particles could have a prompt and

prolonged access to vessel walls, synovial lining cells, and joint fluid.

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