THE FINE STRUCTURES AND THE PERMEABILITIES OF VESSELS AND CELLS, with Special Reference to the Lymphatic System; and other papers.

A collection submitted for the degree of Doctor of Science in the University of Adelaide.

by

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I N D E X

9. "The fine structures, properties and permeabilities of the endothelium. How these determine the functioning of the lymphatic system." In "New trends in Basic Lymphology." Ed. J. Collette; Birkhauser Verlag; Basel. Stuttgart (1967).

10. "The fine structures and permeabilities of lymphatics under some pathological conditions." In "New Trends in Basic Lymphology." Ed. J. Collette; Birkhauser Verlag; Basel. Stuttgart (1967).

11. "Electron microscopical observation on the dilated lymphatics in oedematous regions and their collapse following hyaluronidase administration." Brit. J. Exp. Path. (1967) 48, 680 - 688.

12. "The dilation of lymphatics by oedema and their collapse following hyaluronidase." In "Progress in Lymphology II." Ed. by M. Viamonte et al., Georg Thieme, Stuttgart (1970).

13. "The treatment of acute lymphoedema with pantothenic acid and pyridoxine; an electron microscopical investigation." (With M. Földi and O.T. Zoltan). Lymphology (1969) 2, 63 - 71.

14. "How the lymphatic system overcomes the inadequacies of the blood system." In "Progress in Lymphology II" Ed. by M. Viamonte et al., George Thieme, Stuttgart (1970).

15. "Editorial. How the lymphatic system works." Lymphology (1968) 1, 77 - 80.

16. "An electron microscopical demonstration of the permeability of cerebral and retinal capillaries to ions." Experientia (1969) 25, 845 - 847.

SECTION II: On the Cytoplasmic Vesicles

a). How they transport material

17. "The Brownian movements of pinocytic vesicles." J. Roy. Microscop. Soc. (1963) 82, 257 - 261.

18. "Pinocytic vesicles: An explanation of some of the problems associated with the passage of particles into and through cells via these bodies." Med. Res. (1963) 1, 58 (abstract).

19. "The uptake of particulate lipid preparations by macrophages in vitro: an electron microscopical study." (With A.J. Day) Quart. J. Exp. Physiol. (1966) 51, 1 - 10.

20. "Endocytosis: The different energy requirements for the uptake of particles by small and large vesicles into peritoneal macrophages." J. Microscopy (1969) 90, 15 - 32.

21. "The dimensions and numbers of small vesicles in endothelial and mesothelial cells and the significance of these for endothelial permeability." J. Microscopy (1969) 90, 251 - 269.

22. "The Transportation of large molecules, by the small vesicles, through the endothelium." In "Progress in Lymphology II." Ed. by M. Viamonte et al., Georg Thieme, Stuttgart (1970).

b). Other Observations on Vesicles

23. "The formation of membranes around micro-organisms and particles injected into amoebae: support for the "reticulosome" concept." (With T. Savanat). Aust. J. Exp. Biol. Med. Sci. (1966) 44, 111 - 122.

24. "The functional development of the reticulo-endothelial system. II. The histology of blood clearance by the fixed macrophages of foetal rats." (With P.C. Reade). Immunol. (1965) 9, 61 - 66.

25. "An electron microscopical study of the uptake of foreign particles by the livers of foetal and adult rats." (With P.C. Reade). Brit. J. Exp. Path. (1965) 46, 473 - 480.

26. "The passage of ferritin into jejunal epithelial cells." Experientia (1967) 23, 370 - 374.

APPENDIX I: Miscellaneous Papers involving the Electron Microscope.

a). Technical procedures for lipids.

27. "Some observations on the fixation and staining of lipids." J. Roy. Microscop. Soc. (1963) 81, 235 - 238.

28. "Some observations on the electron microscopy of lipids." J. Roy. Microscop. Soc. (1967) 87, 463 - 473.

b). Other technical problems

29. "The displacement of ferritin particles by the microtome knife." J. Microscopie (1962) 1, 335 - 342.

30. "Quantitative electron microscopy: methods for dry-mass determination, avoiding errors introduced by irradiation changes and contaminating films." J. Ultrastructure Res. (1967) 18, 600 - 604.

c). Sundry papers

31. "An electron microscopical study of a Na^+ and K^+ stimulated ATPase system. Further evidence for the role of this enzyme in sodium and potassium transport." (With J.B. Charnock and L.J. Opit. Biochim. Biophys. Acta (1966) 126, 350 - 360.

32. "Recurrent rhabdomyolysis precipitated by alcohol. A case report with physiological and electron microscopical studies of skeletal muscle." (With R.M. Douglas, J.D. Fewings and R.F. West). Austral. Ann. Med. (1966) 15, 251 - 261.

33. "Electron microscopical observations on the organisation of artificial pulmonary thromboemboli in the rabbit." (With N.G. Ardlie and C.J. Schwartz). Brit. J. Exp. Path. (1967) 48, 501 - 507.

34. "Transformation of the granules of intestinal polymorphonuclear eosiniphils by helminthic infestation." J. Path. Bact. (1968) 95, 299 - 301.

APPENDIX II: Studies on Australian Aborigines

a). Haematology

35. "The haematology of the Central Australian aborigine. I. Haemoglobin and erythrocytes." Austral. J. Exp. Biol. Med. Sci. (1958) 36, 23 - 38.

36. "The haematology of the Central Australian aborigine. II. Leukocyte and eosinophil counts. Casoni Tests." Austral. J. Exp. Biol. Med. Sci. (1959), 37, 481 - 488.

37. "The haematology of the Central Australian aborigine. III. Arneth counts and lymphocyte maturity counts." Austral. J. Exp. Biol. Med. Sci. (1959) 37, 517 - 522.

38. "The haematology of the Central Australian aborigine. IV. Erythrocyte constants and sedimentation rates." Austral. J. Exp. Biol. Med. Sci. (1960) 38, 37 - 46.

b). Blood pressures and other factors possibly relating to atherosclerosis.

39. "Blood pressures in Australian aborigines." Med. J. Austral. (1959) i, 627 - 633.

40. "Serum cholesterol and phospholipid levels of Australian aborigines." (With C.J. Schwartz, A.J. Day, and J. Andrew Peters) Austral. J. Exp. Biol. Med. Sci. (1957) 35, 449 - 456.

41. "Serum cholesterol levels in atherosclerotic subjects and in the Australian aborigines." (With C.J. Schwartz) Med. J. Austral. (1958) ii, 84 - 86.

42. "Atherosclerosis and the serum mucoprotein levels of the Australian aborigine." (With C.J. Schwartz) Austral J. Exp. Biol. Med. Sci (1958) 36, 117 - 120.

43. "The serum magnesium levels of Central Australian aborigines, normal and atherosclerotic Europeans." (With J.S. Charnock, and C.J. Schwartz). Austral. J. Exp. Biol. Med. Sci. (1959) 137, 509 - 516.

c). Sundry papers

44. "A blood group genetical survey in Australian aborigines at Haast's Bluff, Central Australia." (With R.T. Simmons, N.H. Semple and J.B. Cleland) Am. J. Phys. Anthrop. (1957) 15, 547 - 554.

45. "Serum proteins of some Central and South Australian aborigines." (With G.K. Wilkinson, A.J. Day, and J. Andrew Peters) Med. J. Austral. (1958) ii, 158 - 160.

46. "Observations on serum antibodies in aborigines of the Northern Territory." (With P. Warner, and M. Beech) Med. J. Austral. (1957) ii, 857 - 858.

NOTES ON THE INDIVIDUAL PAPERS

The main sources of information leading to the work presented here have been mentioned in the Preface; the detailed references are discussed in the individual papers. Hence only a few will be mentioned here, when this is necessary to round-out the descriptions of the new findings. The details of the assistance given by collaborators will be indicated, where their names appear on the papers. Other assistance, which was not great enough for the paper to be a joint one, e.g. the donation of various substances, is recorded in the Acknowledgements of the individual papers.

Section I

Papers 1 - 4 record investigations into the fine structure and permeabilities of small lymphatics, in different regions, under various normal and pathological conditions; they are reviewed in paper 5. (In paper 1, Lord Florey initiated the project, performed about a quarter of the actual investigations and was responsible for about half of the paper itself.) It was shown how the fragile nature of the bonds uniting the cells forming the small lymphatics allows the intercellular junctions to become opened during tissue activity or injury. This permits many large molecules, etc., to enter the vessels; since the junctions close when the tissues are compressed, most of the lymph is trapped in the vessels and forced into the collecting lymphatics. The normal, slow passage of large molecules through the endothelium everywhere, in both the blood and lymph systems, was shown to be almost certainly caused by their passage in small, smooth, cytoplasmic vesicles. It was also demonstrated that one cannot speak of the "permeability of endothelium" but that it is necessary to consider

the endothelial site, the particular path through the barrier, the substance involved, and the various conditions present at a particular time. One must consider the "fine permeabilities" of the vessel walls just as one must consider their "fine structures".

Paper 6 is a study of the passage of ions through endothelial and mesothelial barriers. The technique was one initially used by Florey (1926), but adapted to the electron microscope. Pairs of mutually precipitating ions were allowed to diffuse through the tissues in opposite directions. The deposits in the endothelial and mesothelial barriers were principally in the intercellular junctions. This experimentally demonstrated that even closed junctions are quite permeable to small molecules. These junctions probably correspond to Pappenheimer's pores (1953). (Similar results have also been independently obtained by groups at Harvard and in Japan.)

Mayerson (1963) considered the two great problems in understanding the functioning of the lymphatic system were to explain how large molecules enter the vessels and how they are retained in them. Papers 1 - 5 indicate how material enters the small vessels. Paper 7 shows that the larger, better supported, collecting lymphatics have closed endothelial junctions, which prevent the large molecules escaping, but which are still permeable to small molecules (paper 8). Thus the entrance and retention of lymph are explained, together with why lymph is concentrated as it passes centrally. These results are reviewed in papers 9 and 10.

Paper 11 demonstrates experimentally that Pullinger and Florey (1935) were correct when they said that lymphatics were dilated in oedematous regions by the increased tensions in the connective tissue fibrils attached to their walls. It disproves a claim by Virágh et al. (1966) that they are dilated

because the endothelium actively transfers fluid into them, and also confirms the findings of Leak and Burke (1966) that small fibrils attach to the cells. The importance of these fibrils for the opening of the junctions is emphasised. It was also realised how transient increased external pressures could compress the tissues, reduce the tensions in the fibrils, and allow the lymphatics to be collapsed by the tissue pressures - thus sealing the junctions and expelling the lymph into the collecting vessels. Paper 12 reviews these and earlier findings relating to junctions and discusses their functioning in greater detail. Paper 13 confirms these results and suggests a possible explanation for the effectiveness of a therapy for acute lymphoedema. (The therapy was devised by Prof. Földi, the animal work was performed by Dr. Zoltan, and the experiment was suggested, the electron microscopy performed and the results analysed by myself.) The whole of papers 1 - 13 are reviewed in paper 14; a simplified version is presented in paper 15.

Paper 16 applies the techniques for training ions used in papers 6 and 8 to the retinal and cerebral capillaries. It is shown that although the junctions are impermeable to large molecules, hence forming part of the blood-brain and blood-retinal barriers, they are permeable to ions.

Section II

a) This part is concerned with the way in which large molecules are transported by the small, smooth cytoplasmic vesicles. It was shown by Gordon and King (1960) and others that large molecules and small particles can be taken up by cells in the absence of cellular activity; large particles need cellular energy to be ingested (Karnovsky, 1962). This suggested three things.

Firstly that the large molecules and small particles might be entering small vesicles, secondly that the small vesicles could be moved about the cell by Brownian Motion, and thirdly that this may be how small vesicles transport large molecules across the endothelial cells. Observations showed that the Brownian movements of fairly small vesicles (paper 17) seem sufficiently great to account for this. Further implications of these ideas were presented in abstract form (paper 18). It is shown in papers 19 and 20 that the small vesicles do indeed take up material, in approximately normal amounts, in the absence of cellular activity and can also coalesce to form one kind of large vesicle. (In paper 19, Dr. Day provided the material and performed the measurements of radio-activity; the electron microscopy and the analysis of the parts of the work relating to the energy requirements of the different types of vesicles were performed by myself.) In paper 4 also, some evidence was presented that material can pass through lymphatic endothelium via the small vesicles in the absence of cellular activity. (This was shown, in much greater detail, for blood capil ary endothelium by Jennings and Florey (1967).)

It therefore seemed fairly clear that the uptake and transportation of material by the small vesicles does not require cellular energy. It also seemed highly probable that Brownian motion was the only force which could account for this. Shea and Karnovsky (1966) showed theoretically that Brownian motion could easily be sufficient to move the vesicles across the cells, but they considered that some other force would be needed to move them away from the plasma membranes. Paper 21 gives more details of the numbers and dimensions of the vesicles. It is shown that Shea and Karnovsky neglected the fact that

vesicles are attached to the plasma membranes by stalks, so that they are released a considerable distance away from the membranes. This disposed of the need to postulate another force as Shea and Karnovsky did; hence Brownian motion can account for the release of the vesicles as well as for their movements. (The formation of the stalks can also be explained by Brownian movements - paper 21.) The application of the results of Grotte (1956), Mayerson (1963) and Jenkin (1964) to the results of paper 21 allow one to calculate the average time that a vesicle is attached to a membrane, its average free life and other parameters. Papers 17 - 21 are reviewed in paper 22, where some other conclusions relating to selectivity are presented.

b) This part contains miscellaneous papers on vesicles. Paper 23 records observations on how membranes form around particles injected into amoebas; "pre-membraneous" particles like the reticulosomes of Pollak and Shorey (1964) were observed. (The problem was suggested and the microinjections performed by Dr. Savanat; the electron microscopy and its interpretation were by myself.) Papers 24 and 25 concern the uptake and subsequent fate of various particles, including bacteria, by the different cells of the liver. (The animal work and light microscopy was performed by Dr. Reade, the electron microscopy by myself.) The passage of ferritin into the intestinal epithelial cells is recorded in paper 26, showing that some proteins can enter the body in this way; this has important implications both for the pathogenesis of intestinal allergies and for lipid absorption.