

## REFERENCES

1. Arey, L.B. (1968) :

Human Histology, A text book in outline form 3rd edition, P 152-157.

Saunders Company, Philadelphia, London and Toronto.

2. Banks, W.J. (1974) :

Histology and comparative organology a text atlas. P. 136-141.

The Williams, Wilkins Company, Baltimore.

3. Bloom, W. and Fawcett, D.W. (1986) :

A Text book of Histology. II edition, P. 464-478.

Sounders company, Philadelphia, London and Toronto.

4. Blue, J.; and Weiss, L. (1981) :

Vascular pathways in non sinusoidal red pulp. An electron microscopic study of the cat spleen.

The American Journal of Anatomy, 161 : 135-168.

5. Blue, J. et al., (1981) :

Species variations in the structure and function of the marginal zone an electron microscopic study of cat spleen.

Am. J. Anat. 161 (2) : 169-187.

6. Bradbury, M.A. (1973) :

Hewer's Textbook of Histology for medical students.  
4th edition. P 210-215.

The English language book society and Williams  
Henemann medical books LTD. London.

7. Braithwaite J.L. and Adams, D.J. (1957) :

The venous drainage of the rat spleen. Journal of  
Anatomy, vol. 91, 352-357.

8. Brown, E. and Dellman, H. (1976) :

Text book of veterinary Histology. P. 175-184.  
Lea and Febiger Co. Philadelphia.

9. Brozman, M. (1985) :

Anatomical pathways from the white pulp to the red  
pulp in human spleen. Acta Anatomica, 121 : 189-193.

10. Bruyn, P.P.H. and Cho, Y. (1974) :

Contractile structures in endothelial cells of  
splenic sinusoids.

Journal Ultrastructure Research, 49 : 24-37.

11. Burke, J.S. and Simon, G.T. (1970) :

Electron microscopy of the spleen I-Anatomy and  
microcirculation.

American Journal of pathology , vol (58) : No (1) :  
127-138.

12. Carr, I. (1972) :

The fine structure of microfibrils and microtubules in macrophages and other lympho reticular cells in relation to cytoplasmic movement.

Journal of Anatomy, 112, (3) : 383-389.

13. Chen, L.T. and Weiss, L. (1972) :

Electron microscopy of the red pulp of human spleen.

Am. J. Anat., 134 : 425-458.

14. Chen, L.T. and Weiss, L. (1973) :

the role of the sinus wall in the passage of erythrocytes through the spleen.

Blood, vol. (41), No. (4) ; 529-537 .

15. Cho, Y. and De Bruyn, P.H. (1975) :

Passage of red blood cells through the sinusoidal wall of the spleen .

Am. J. Anat., 142 : 91-106.

16. Clark, G.; Coalson, R.E. and Nordquist, R.E. (1973) :

Staining procedures 3rd edition p. 1-71.

Williams and wilkins company.

17. Copenhaver, W.M. and Johnson, D.D. (1958) :

Baily's text book of Histology, fourteenth edition, P. 249-273.

Balitmore, the Williams and Wilkings Compnay.

18. Dawson, R.M.C.; Elhott, D.C., Elliott, W.H. and Jones, K.M. (1969) :  
Data for biochemical research. 2nd edition.  
Clarendon press, Oxford.
- X 19. De-Bruyn, P.H., Cho, Y. (1974) :  
Contractile structures in endothelial cells of  
splenic sinusoids. J. Ult. Res. 49 : 24-33.
20. Drury, R.A.B. and Wallington, E.A. (1980) :  
Carlton's Histological technique 4th edition.  
Oxford-london.
21. Downey, H. and Weiden reich F. (1912) :  
Uber die Bildung der lymphocyten in lymph drusen und  
milz. Arch. F. Mikr. Anat. 80 : 306-310.  
(cited by Lashin, 1981 , M.Sc. Thesis, Faculty of Medicine,  
Ain Shams University ).
22. Fujitta, T.; Kashimura, M. and Adachi, K. (1985) :  
Scanning electron microscopy and terminal  
circulation.  
Expertientia, 41, 167-178.
23. Galindo, B. and Freeman, J.A. (1963) :  
Fine structure of splenic pulp.  
Anat. Rec. 147 ; 25-41.
24. Galindo, B. and Imaeda, T. (1962) :  
Electron microscopic study of the white pulp of the  
mouse spleen.  
Anat. Rec. 143 : 399-415.

25. Graf, R. and Schluns, J. (1979) :

Ultrastructural and histochemical investigations of the terminal capillaries in the spleen of the carp (*Cyprinus carpio*).

The Cell Tissue Research, 196, 239-306.

26. Greep, R. and Weiss, L. (1981) :

Histology, 3rd edition, P. 445-475.

McGraw-Hill Book company, New York, St.

Louis, San Francisco, Toronto, London, Sydney.

27. Ham, A.W. and Cormack, D.H. (1980) :

Histology, 8th edition, p. 355-366.

J.B. Lippincott Company Philadelphia and Toronto.

28. Hartwig, H. and Hartwig, H.G. (1985) :

Structural characteristics of the mammalian spleen indicating storage and release of red blood cells.

Aspects of evolutionary and environmental demands.

Experientia, 41 : 159-163.

29. Hebel, R. and Stromberg, M.W. (1976) :

Anatomy of the laboratory rat. p. 121-118.

The Williams and Wilkins Company Baltimore.

30. Heusermann, U. and Stutte, H.J. (1974) :

Intracellular junctions of sinus lining cells in the human spleen.

The cell Tissue Research, 151 : 337-342.

31. Heusermann, U. and Stutte, H.J. (1975) :

Comparative Histochemical and electron microscopic studies on the sinus and venous walls of the human spleen with special reference to the sinus-venous connections.

The Cell Tissue Research, 163, 519-533.

32. Heusermann, U. and Stutte, H.J. (1977) :

Electron microscopic studies of the innervation of the human spleen.

The Cell Tissue Research, 184-225-236.

33. Hirasawa, Y. and Tokuhiro, H. (1970) :

Electron microscopic studies on the normal human spleen, especially on the red pulp and the reticuloendothelial cells.

Blood, Vol. 35, No. 2 (February), 201-212.

34. Junque, L.C. and Carneiro, J. (1983) :

Basic Histology, 4th edition p. 300-306.

Lange Medical Publication, LosAltos California.

35. Karlsson, U and Schultz, R.L. (1965) :  
Fixation of the central nervous system for electron microscopy by aldehyde perfusion-preservation with aldehyde perfusates versus direct perfusion with osmium tetroxide with special reference to membranes and the extra cellular space.  
J. Ultrastructural Res. 12-160.
36. Kobothe, L.L. (1939) :  
Über das Gitter Fasengerüst der roten Milzpulpa mit einem. Beitrag zu ihrer Gefäß Struktur und Blutdurchströmung. Beitr. Zur. Path. Anat. 103, 11-29  
(cited by Raflha, 1977, M.Sc. thesis, Faculty of Medicine, Ain Shams University).
37. Kyber, E. (1870) :  
Über die Milz des Menschen und einiger Säugetiere.  
Arch. Mikr. Anat. 6, 540-570.  
(cited by Braithwaite and Adams, 1956, Nature, 198, 1178-1179).
38. Leeson, G.R.; Leeson, T.S. and Papara, A. A. (1983) :  
Text book of Histology, 4th edition p. 277-290.  
W.B. Saunders company, Philadelphia London, Toronto Mexico city, Rio De Janeiro Sydney, Tokyo, Igaku-Shoin Saunders.
39. Luft, J.H. (1961) :  
Improvements in epoxy resin embedding methods.  
J. Biochem. Cytol 9 : 105

40. Marshall, A.J., parkers, T.J. and Haswell, W.A. (1978) :  
Text book of zoology vertebrates, 7th edition, p.  
654-682.  
English Language Book Society and Macmillan.
41. Mc Cusky, R.s. and McCusky, P.A. (1985) :  
In vivo and electron microscopic studies of the  
splenic micro vasculature in mice.  
Expertientia, 41 : 179-187.
42. Mc Laughlin, C.A. and Chiasson, R.b. (1979) :  
Laboratory anatomy of the rabbit.  
2nd edition, p. 35-46.  
Brown company publisher, Dubuque, Iowa.
43. Miller, M.E.; Christensen G.C. and Evans, H.E. (1964):  
Anatomy of the dog, P. 458-461.  
W.B. Saunders Company, Philadelphia, London
44. Mollenhaver, H.H. (1964) :  
Plastic embedding mixtures for use in electron  
microscopy.  
Stain Technology, 39 111-114.
45. Palade, C.E. (1952) :  
A study of fixation for electron microscopy.  
J. Exp. Med. 95 : 285 - 297.



46. Reilly, F.D.; McCuskey, R. and Meineke, H.A. (1975) :

Studies of the hemopoietic microenvironment.  
VIII-Adrenergic and cholinergic innervation of the  
murine spleen.

Anat. Rec., 185 : 109-118.

47. Reilly, F.D. (1985) :

Innervation and vascular pharmacodynamics of the  
mammalian spleen.

Experientia, 41 : 187-192.

48. Rhodin, J.A.G. (1974) :

Histology, A text book and Atlas 3rd, edition, P.  
400-415.

New York Medical collage, New York Oxford University  
press, London-Toronto.

49. Roberts, D.K. and Latta, J.S. (1964) :

Electron microscopic studies on the red pulp of the  
rabbit spleen.

Anat. Rec. 148 : 81-85.

50. Ross, M.H. and Reith, E.J. (1985) :

Histology, A text and Atlas, 1st edition, p. 315-321.

Harper and Row publishers, J.B. Lippincott company,  
New York, Cambridge Philadelphia, San Francisco,  
London, Mexico city, Sao Paulo, Singapore, Sydney.

51. Sabatini, D.s.; Bensch, K. and Barret (1963) :

Cytochemistry and preservation of cellular ultrastructural and enzymatic activity by aldehyde fixation.

J. Cell Biol. 17 : 19-24.

52. Saito, H.; Yokoi, Y.; Watanabe, s.; Tajima, J. Kuroda, H. and Namihisa, T. (1988) :

Reticular meshwork of the spleen in rats studied by electron microscopy.

Am. J. Anat. 181 : 235-252.

53. Sasou, S.; Satodate, R. and Katsura, S. (1976) :

The marginal sinus in the perifollicular region of the rat spleen.

The cell Tissue Research, 172 : 195-203.

54. Snell, R.s. (1972) :

Clinical embryology for medical students, p. 255, 2nd edition, little, Brown and Company. Boston

55. Snell, R.S. (1984) :

Clinical and Functional Histology for Medical Students. 3rd edition p. 349-359.

Little Brown company, Boston, Toronto.

56. Snodgrass, M.J. (1971) :

Cytochemical and functional aspects of the sinus lining cells of the rabbit spleen.

Journal of Reticuloendothelial Society, 10, 184-199.

57. Snoge, S.H. and Jano, V.H. (1981) :

Human microscopic anatomy, 1st edition, p.230-245

McGraw-Hill company, New York, St Louis, San Francisco  
Sydney Tokyo, Toronto.

58. Snook, T. (1964) :

Studies on the perifollicular region of the rat's spleen.

Anat. Rec. 148 : 149 - 159.

59. Steinman, R.M.; Adams, J.C. and Cohn Z.A. (1975) :

Identification of a novel cell type in peripheral lymphoid organs of mice.

Journal of experimental Medicine, vol. 141, p.  
804-820.

60. Tehver, J. and Grahame, T. (1930) :

The capsule and trabeculae of the spleen of domestic mammals.

Journal of Anatomy, 65 : 473-481.

61. Thomas, C.E. (1967) :

An electron and light microscope study of sinus structure in perfused rabbit and dog spleens .

Am. J. Anat. 120 : 527-552.

62. Veerman, A.J.P. and Ewijk, W.V. (1975) :

White pulp compartments in the spleen of rats and mice, a light and electron microscopic study of lymphoid and non lymphoid cell types in T and B areas. Cell Tissue Res. 156, 417-441.

63. Vidyarthi, R.D. (1971) :

A text book of zoology, eleventh reprint edition p. 531-535, Agrasia Publishers. Daresi 2, Agara 4.

64. Warwick, R. and Williams, P.L. (1983) :

Gray's Anatomy 35 edition, p. 773-799.  
Longman's Green, London.

65. Weibel, E.R. and Palade, G.E. (1964) :

New cytoplasmic compartements in arterial endothelium.

J. Cell. Biol. 23 : 101-112.

66. Weiss, L. (1957) :

A study of the structure of splenic sinuses in man and in the albino rat with the light microscope and the electron microscope.

J. Biophys. Biochem. Cytol. 3 : 599-609.

67. Weiss, L. (1962) :

The structure of fine splenic arterial vessels in relation to hemoconcentration and red cell destruction.

Am. Journal of anat., 111 : 131-174.

68. Weiss, L. (1963) :

The structure of intermediate vascular pathways in the spleen of rabbits.

Am. Journal of Anat., 113, 51-91.

69. Weiss, L. (1973) :

The development of the primary reticulum in the spleen of human fetuses (38 to 57 mm crown rump length).

Am. Journal Anat., 136 , 315-338.

70. Weiss, L. (1973) :

Histology, 3rd edition, 445-476, McGraw Hil Book company, New York, St Louis, San Francisco ,Tronto London,Sydney .

71. Windle, W.F. (1960) :

Textbook of Histology, 4th edition, p 202-210.

McGraw Hill company, New York, St. Louis, San Francisco, Toronto, London, Sydney.

B stain for about 2 minutes, then washed in tap water for 10 minutes.

3. Sections were put into ascending grades of alcohol (50%, 70%, 90% and 100%) for 2 minutes in each.
4. The sections were cleared in two changes of xylol each for about 2 minutes.
5. Sections were mounted with canada Balsam, and left to dry in an oven at 37°C.

**\* Results :**

Nuclei → blue, blue-black  
cytoplasm → Shades of pink  
Collagen → light pink  
Red blood cells → red-bright orange.

## II. Masson's Trichrome Stain : (Drury and Wallington,1980)

For demonstration of the collagenous tissue and muscle fibers.

### \* Preparation :

#### a. Masson's Stain :

##### - Cytoplasmic stain :

1 % Ponceau dexylidine in 1% acetic acid (2 parts).

1% Acid Fuchsin in 1% acetic acid (1 part) .

##### - Differentiation and Mordant :

1% Phosphomolybdic acid in distilled water

##### - Fiber stain :

2 % aniline blue dye .

#### b. Weight's Iron Haematoxylin :

Basic fuchsin	2 gm.
Rosorcin	4 gm.
Distilled water	200 ml.
30% Ferric chloride	25 ml.
95% alcohol	200 ml.
Concentrated Hcl	4 ml.

2 gm.basic fuchsin and 4 gm.resorcin were added to 200 ml. distilled water in a beaker, then boiled and when both ingredients had been dissolved and while still boiling, 25 ml. of 30% ferric chloride was added. The stirring and boiling were continuous for a further 2-5 minutes untill the coarse precipitate was ceased to form . The solution was filtered and the filterate was discarded.

The precipitate was dried on the filter paper by leaving it over night in the incubator. The filter paper and its content were returned to the beaker which contained small amount of residual precipitate. 200 ml. of 95% alcohol was added and the precipitate was dissolved by gentle heating in water bath or on an electric hot plate.

When dissolved, the solution was left to cool then filtered and the volume was restored to 200 ml. with 5% alcohol. 4 ml. conc. HCl was added and bottled with a tightly fitting stopper. This solution could be used for many months.

**\* Technique :**

1. Sections were put in water for 3 minutes.
2. Stained with weigert's iron haematoxylin for 15-20 minutes.
3. Sections then were washed well in tap water then rinsed in distilled water.
4. Stained in the red cytoplasmic stain for 5-10 minutes.
5. Then rinsed in distilled water.
6. Sections were differentiated in 1% phosphomolybdic acid, to decolourized the collagen and keeping the red colour of the muscle fibers red blood cells and fibrin.
7. The sections then rinsed in distilled water.
8. The sections were counter stained in aniline blue for 2-5 minutes.



9. Wash well in 1% acetic acid for at least one minute.
10. Then the sections were blotted, dehydrated in absolute alcohol, cleared in xylene and mounted in a synthetic resin medium.

Results :

Nuclei	→	black
Muscle, red blood cells, fibrin	→	red
Collagen	→	blue .

### III. Orcein Stain : (Drury and Wallington, 1980)

For demonstration of elastic fibers.

#### \* Preparation :

Orcein (synthetic)	1 gm.
80% alcohol	100 ml.
Conc. Hcl	1 ml.

#### \* Technique :

1. Sections were dewaxed in two changes of xylol, each change for 5 minutes then were taken to water via descending grades of alcohol.
2. Placed in closed Jar of the stain for  $\frac{1}{2}$ -2 hours, at room temperature.
3. Wash well in 70% alcohol, the staining of collagen might be removed by treatment with 1% acid alcohol.
4. Washed, well in tap water.
5. Counter stain the nuclei lightly with methylene blue.
6. Dehydrate, clear, and mount in synthetic resin medium.

#### \* Result :

Elastic fibers	→	dark brown
Nuclei	→	blue

IV. Van Geison's Stain: (Drury and Wallington, 1980)

Used for staining collagenous tissue and muscle fibers.

\* Preparation :

- Van Geison solution .

- . Saturated aqueous picric acid                      100 ml.
- . 1% fuchsin in distilled water                      5-10 ml.

- Weighert's iron haematoxylin :

Had described previously.

\* Technique :

1. Sections were put in water for 3 minutes.
2. Nuclei were stained with weighert's iron haematoxyline for 20-30 minutes.
3. Pushed in tap water and followed by a rinse in distilled water.
4. Put in Van Geison solution for 2-5 minutes.
5. Rinse in distilled water.
6. Dehydrated in absolute alcohol, cleared in xylene and mounted in asynthetic resin medium.

Results :

Nuclei                      →      brown black to black  
Collagen                      →      deep red  
Muscle, cytoplasm, red blood, fibrin → yellow

V. Gordon and Sweets Reticulin Stain : (Drury and Wallington, 1980) .

For demonstration of reticular fibers .

\* Preparation :

Silver solution : add strong ammonia to 5 cm<sup>3</sup>. of 10.2% silver nitrate drop by drop untill the resulting precipitate was just dissolved. Add 5 cm<sup>3</sup>. of 3.1% sodium hydroxide and redissolve the precipitate with a few drops of ammonia. Dilute up to 50 cm<sup>3</sup> with distilled water.

\* Technique :

1. Sections were put in water for 3 minutes.
2. Oxidized for 1-5 minutes in 0.5% potassium permanganate (47.5 cm<sup>3</sup>) + 3% sulphuric acid (2.5 cm<sup>3</sup>).
3. Washed briefly in water.
4. Bleached in 1% oxalic acid.
5. Rinsed in distilled water followed by through washing in tap water.
6. Sensitized in 2.5% iron alum for ½-2 hours .
7. Washed throughly with two or three applications of distilled water.
8. Covered with the silver solution for 10-30 second untill sections became transparent.
9. Washed well with distilled water.
10. Reduced with 10 percent neutral formalin for 1-2 minutes .
11. Washed in tap water followed by distilled water.