Introduction to Protozoa and Fungi in Periodontal Infections

A Manual of Microbiological Diagnosis and Nonsurgical Treatment

By

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All photomicrographs were taken with an Olympus OM-2S camera mounted on an Olympus BH2 microscope. Phase contrast condenser and 100x objective lens (oil immersion) together with an Olympus NFK 3.3 photo eyepiece were used unless otherwise stated. This gives a page magnification of approximately x1,000.

ILLUSTRATION FOR CHAPTER II

Two very large amœbæ, pressed tightly against each other, almost entirely fill the field. The endoplasm and ectoplasm are not well differentiated. The nuclei are clearly visible, a circle with a dot offset from the centre. Thickenings on the outer ring represent chromosomes. Two may be seen in the left hand example. Both amœbæ have many food vacuoles. The dense matter in the larger vacuoles are the partly digested remains of the nuclei of leucocytes. Small dense black dots within the cytoplasm are granules of ingested haemoglobin. Pale grey granules within vacuoles are fragments of ingested leucocyte cytoplasm. A third (smaller) amœba, in which the nucleus cannot be seen, is immediately below the left-hand amœba, but touching both. A fourth amœba is half way into the field of view at the lower border of the picture, just to the right of centre.
1. Two amoebae occupy the centre of the field. Each contains a nucleus (circle with a dot in the middle). Both have many food vacuoles. A saturated solution of Modified Torre's Powder has just diffused to the area and the smaller of the two amoebae is showing signs of shrinkage.

2. Within a minute the two amoebae are shrivelled, shrunken masses which appear to have a protective pellicle (precyst). The internal structure is no longer apparent.
3. Half an hour later the amœbæ have partly recovered and are showing signs of vitality by moving sluggishly again.

Aloe Vera Solution: Series Two.
Cell Membrane Lysis.

4. An amœba is seen in the centre of the field. A solution of Aloe Vera is just entering the field from the “river estuary” immediately above the amœba.
5. On contact the cell membrane starts to lyse.

6. Within a minute the cell membrane has dissolved and the cell contents are floating away from the ruptured parasite, however, the nucleus remains clearly defined.
Modified Torren's Powder: Series Three.
Reverse Osmosis.

(Wild Heerbrugg phase contrast microscope/Pentax SP 500. Special Setup. Page magnification approximately x1,000. Reprocessed from colour slides.)

7. An amoeba, with many large fluid filled vacuoles is seen adjacent to a white granular mass. The vacuoles rapidly fill with fluid which is then discharged through the cell membrane. The process continues for three quarters of an hour, during which the amoeba, apparently unable to escape, loses fluid and shrinks.

8. Ultimately the membrane is ruptured and fragments of cytoplasm separate from the carcass. Both are attacked by spirochaetes, which seem to be unaffected by the presence of the mixture of baking soda and salt.
9. A nest of three amoebae, pressed tightly together, is central to the field. The smallest amoeba is about the size of a red blood cell, of which there are a number in this field. A red blood cell may be seen to the right of the "tail" of the large amoeba. This amoeba has a clearly defined nucleus (circle with a dot on the middle). It is feeding on the remains of a leucocyte, which is out of focus at the upper right hand end of the "tail" of the amoeba. At the junction of the two cells a dark wavy line may be seen. It terminates in a grey disk. All of the remains of the leucocyte nucleus will ultimately be reconstituted into a disorganised mass of partly digested nuclear material in a food vacuole.
10. Three large amoebae, the lower one is feeding on a leucocyte. The remains of the leucocyte nucleus are about to be completely sucked into the amoeba. Peristaltic waves along the thick black line rapidly move the leucocyte nuclear material into the food vacuole. The nucleus of the amoeba may be clearly seen in the middle (non-feeding) amoeba but with less ease in either of the other two.
11. An amöeba, with a clearly defined nucleus is seen sucking the hæmoglobin from an erythrocyte. The thin black line of hæmoglobin terminates in a black dot within the amöeba cytoplasm. The material does not seem to be enclosed in a vacuole.
12. An amoeba in the centre of the field may be differentiated from the surrounding leucocytes. The food vacuoles and nucleus of the amoeba may be seen. This amoeba is feeding on a red blood cell, as evidenced by the undulating thin black line running vertically from the black disk (erythrocyte) stuck to the cell membrane of the amoeba. (40x objective lens, page magnification approximately x400).
13. An amoeba, trailing several red cells and the remains of a white cell, thrusts itself between two epithelial cells.

14., 15., 16. Moving relentlessly forward, the amoeba dislodges an epithelial cell.
ILLUSTRATIONS FOR CHAPTER IV

Identification and function of various components of a typical microscope.

Illustrations courtesy of Olympus Optical Co. Ltd.

Light path selector knob
The knob can be operated in 3 positions to deflect the light as desired.

Tension adjustment ring
Clockwise rotation increases coarse adjustment tension.

Coarse adjustment knob
Coarse adjustment range: 26mm

Fine adjustment knob
Graduated in increments of 2µ.

Filter mount
Accepts the ND filter holder.

ND filter holder*
Filter diameter 45mm.

Sliding voltage control lever
Voltage varies as the lever is pulled toward the microscope front.
17., 18., 19. Amoebae, recognizable by their unique morphology, ingested remains of leucocytes and erythrocyte, movement and especially the typical nucleus can be differentiated from surrounding plaque (peas and spaghetti) and leucocytes (cells with one, two, three or four lobes to the nucleus and, in live wet mounts, granules dancing in the cytoplasm).
20. A dead amoeba is seen to the left and below the live amoeba. Although dead, the nucleus remains clearly visible. The cytoplasm has lost its hyaline appearance, instead it looks like “Swiss cheese” or an “Aero” chocolate bar in cross section.
21., 22. An amöeba, recognized by its nucleus, is seen interacting with a leucocyte. The granules in the leucocyte may be seen clumped along the area of intercellular contact. The white cell to the left of the amöeba in the first illustration has a paler central section due to thinning of the granules. In the second illustration the granules in the three leucocytes show clumping at the area of contact with the amöeba but marked thinning elsewhere. Degenerating leucocytes are easily differentiated from healthy ones by the density of the granules in the cytoplasm. As the density reduces, the granules move less vigorously and migrate to the periphery of the cell so that the cell appears more translucent.
23. Low Power, Phase Contrast, Pseudo Dark Field. 10x objective lens. Approximate page magnification x100. (Photographically enhanced.) Look for the black oval object in the centre of the field. It has a bright centre. Confirm that you have spotted an amœba by changing to 100x objective lens (next illustration, #24) by comparison of shape and identification of nucleus. (Circle with a dot in the middle. In this case the central karyosome is a small circle, concentric with the outer membrane. Three thickenings of the chromatin on this outer ring are thought to correspond to the positions of chromosomes.) Note the ingested remains of many blood cells. Can you spot other amœbæ in picture #23?

24.
25, 26. Low Power/High Power. Do not confuse erythrocytes, a bright circle with a black centre but no inner bright core, with amoebae at low power. Note the presence of epithelial cells, seen as grey areas with whiter nucleus. The white areas in the low power pictures are composed of the mass of the bacteria in the plaque. The black areas are open spaces in which motile bacteria may be more easily observed at higher magnification.
27. Contrast the hyaline amoeba (single nucleus, circle with a dot in the middle) with the granulated leucocyte (granulocyte/polymorph with multi-lobed nucleus.)

28. Large amoeba recognized by its nucleus, small circle with thickenings; offset central karyosome, a faint circle; ingested food. In a live wet mount of plaque the typical movement of amoebae should leave no doubt about its identity. Nothing else in plaque moves in the same way. Note the white cell (lymphocyte?) below and to the left of the amoeba. The white cell nucleus is larger and more complex, the cytoplasm granulated and clearer. Although some white cells show amoeboïd movement in plaque, it differs in character from that exhibited by the protozoa.
29. A dead amoeba, recognized by its nucleus, differs in appearance from a live amoeba by mottled appearance of the cytoplasm and rupture of the cell membrane. A mat of rods, cocci and some filaments may be seen adjacent to this amoeba. Note the red cell to the left and the white cell and two epithelial cells which just clip the field of view.

30. A degenerating amoeba, centre, recognized by its nucleus. Below it there is a clump of bacteria with cell debris. To the left is a grey blob, ("grease blot"). These two forms are typical of amoebae under adverse environmental conditions. The "grease blot", devoid of any inclusions, has become separated from an amoeba. The latter retains the nucleus and food vacuoles. The "grease blot" may move about randomly.
31. A leucocyte, some filaments, rods and cocci.

32. Blood platelets
33. Trichomonas tenax. One is rounded up in the centre of the field. The other is a pear shaped cell to the right. The main mass, surrounding these two vigorously moving protozoa, are non-moving rods, filaments and cocci (Lepto++, Cocci++). There are two epithelial cells at the left corner of the picture. The black disk with the halo is a grit particle or other unimportant artifact. The count for two trichomonads in the field of view, high power, is +++. 

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34. *Trichomonas tenax* (*p*), large variant. Note flagella at the 12 o'clock position. Spirochetes and motile bacilli are also seen sharply focussed because the lamp on the microscope at full brilliance, directed 100% into the camera, permits a fast shutter speed with high resolution black and white film (ASA 125).

Generally, trichomonads found in the mouth will be labelled as *Trichomonas tenax*, (6-10 microns) but the larger variant might be a transient infection with *Trichomonas vaginalis*, (5-15 microns, but it may reach a length of 30 microns).
35., 36., 37. Candida albicans. Typical appearance in plaque. Note long strands (hyphae), long cells, joined end to end (pseudohyphae), large oval cells (chlamydospores) and small oval cells (yeast cells). Note dense ball in some vacuoles. In a live wet mount these bounce around inside the cell. Note differing density of cells, especially note in #36 and #37 that some cells have shrivelled cellular contents and in some cases the cell membrane is seen to be perforated. This is typical of the appearance following treatment with Nystatin or Ketoconazole.
38. Dense yeast cells with an encapsulated appearance in necrotic tissue. The outline of an epithelial cell is just visible. Swabbing to confirm the identification of such yeast cells may result in surprises. Genus Candida, Candida albicans, or even Penicillium species have all been recorded. Microbiological findings must always be related to clinical findings and patient symptoms. Some infections will necessitate dentist and physician co-operation.
39., 40. Variable appearance of filaments with cocci attached. Branching filaments are Actinomyces, non-branching filaments are Leptothrix. These symbiotic colonies are all referred to as ACs in this text. The filaments illustrated are not Candida or other fungi. Compare with #35-#38.
41. Ropes of Leptothrices.

42. Ropes of Actinomyces
43. Streptococci ........ a string of pearls.

44. Spirochætes and bacilli swimming in open space adjacent to a clump of non-motile rods and cocci.
45. Entamoeba gingivalis resembling multinucleated giant cells.

46. Microscopist’s Shock - A microscopic mite seen in plaque x100.
ILLUSTRATIONS FOR CHAPTER V

Case Four: #47 + #48

47. Radiograph of periapical lesion. After removal of the tooth, the apical granuloma was biopsied for a parasitology report.

48. (Below) Stained slide shows presence of E. gingivalis. The nucleus of one of the two amoebae in this field can be seen in the lower left corner (circle with a large blot in the centre).
Case Five: #49 + #50

49. Stained slide shows amoeba from plaque

50. Stained Slide shows amoeba from tonsillar pus.
Series Two, Case One: #51 + #52

51. Radiograph August 1977

52. Radiograph April 1984.
Series Two, Case Two: #53 + #54

53. Radiograph August 1983

54. Radiograph May 1986
Illustrations

Series Two, Case Three: #55


56. A typical, but extreme example of black hairy tongue. Sometimes this condition is a sequel to the use of antibiotics used to eliminate the protozoa or other infections.
57. A typical pretreatment radiograph. May 1982

58. Following successful elimination of protozoa, a follow up radiograph, February 1985, shows that there has been bone regeneration.
Series Two, Case Four: #59 + #60

59. November 1985

60. March 1986

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Series Two, Case Five: #61 + #62

61. Radiograph 1973. Note loss of bone at the tuberosity, also mesial to the maxillary canine, mesial to the mandibular first molar and cuspid.

Series Two, Case Seven: #63 + #64


64. April 1986. Re-establishment of interdental papilla by 'creeping reattachment'.
65. A light probing technique with a blunt thin probe, such as a Williams probe, is less likely to injure fragile tissue or spread infection.

66. Sublingual saliva is taken with a pair of college tweezers.
Illustrations

67. ... and deposited on a clean microscope slide.

68. A fine probe is inserted vertically to the base of the pocket, and taking care to avoid promoting hæmorrhage, it is rotated ...
69. .......... and lifted clear, together with a small sample of plaque from the base of the pocket.

70. The plaque is quickly transferred to the saliva on the slide, taking care not to agitate the sample.
71. A cover slip is dropped in place ......

72. .... and a pipe cleaner used as a squeegee .......
73. ....... to produce a thin film.

74. The slide is placed on the stage of the microscope ......

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75. ..... the lamp is switched on and put at about half brilliance, the 10x objective is swung into position and the slide can be examined.

76. (Below) Low power (x100), phase contrast, pseudo dark field appearance of plaque. Scan and spot.
77. (Above) Note that the left hand focuses the microscope lens system while the right manipulates the stage traverse controls.

78. Having spotted an amœba (or two, review picture #76), the lens turret is rotated so that a drop of oil may be place on the cover slip (on the bright spot of light which is the field of vision)
79. The 100x oil immersion lens is now swung into position ........

(Did you spot the deliberate mistake in this picture? If not review the text on use of a microscope.)

80. ........ and the diagnosis is confirmed. (Compare with the low power, #76)
81., 82. Following instrumentation in a site known (or suspected) to be infected, it may be advisable to apply a suitable antiseptic (here MA paste is being applied), using a toothbrush, an instrument or a syringe with a canula or a blunt needle, to minimise the chance of iaterogenic spread of infection. In some instances, systemic antimicrobials may also be indicated. Parodontal abscesses can occur following examination of severe periodontal lesions.