Lipids

Dr Phil Bryant, Wales, UK
Lipids

- Lipids are structural components of cell membranes
- Lipids store energy
- Lipids form the basis of hormones
LIPIDS

Simple lipids
- Esters of fatty acids
  - Fats
  - Esters of fatty acids and glycerol

Compound lipids
- Esters of fatty acids and alcohol contain other groups also
  - Phosphatides (lecithin, cephalin, sphingomyelin)
  - Cerebrosides (glycolipids & galactolipids such as kerasine and phrenosine)

Derived lipids
- Composed of hydrocarbon rings and a long hydrocarbon side chain
  - Fatty acids (saturated and unsaturated)
  - Alcohols (sterols such as cholesterol)
Fat cells - adipocytes
Two types of fat cell

- **White fat cell**
  - Most common
  - Unilocular - single, large lipid droplet
  - Large diameter (>100u)
  - Subcutaneous
  - Omentum
  - Mesentery

- **Brown fat cell**
  - Less common
  - Multilocular - many small droplets
  - Common in new-born
  - Around kidney
  - Neck
  - Mediastinum
- Fat cells are thought to develop from fibroblast-like cells.
- Fat droplets coalesce into a large droplet, leaving only a thin rim of cytoplasm.
- Nucleus is usually pushed to the side.
When tissue is fixed and stained, the single large lipid droplet is extracted and the cells look empty.
Identification of lipids

1. Solubility - differentiation of lipids by their solubility in various solvents
2. Examination by polarised light
3. Reduction of osmium tetroxide
4. Demonstration by fat soluble dyes
5. Histochemical methods
1. Solubility

- Keilig’s extraction using fresh human brain, extracted with 3 changes over 24 hours
- **Cold acetone** - simple lipids, neutral lipids & cholesterol
- **Hot acetone** - compound lipids such as cerebrosides
- **Hot ether** - phosphatides such as lecithin and cephalin
- **Hot chloroform and methanol** - all lipids
- Following extraction, blocks hydrated through alcohol to water, frozen cut and stained with Sudan black
2. Examination by polarised light

- Three types of refractility:
  - Isotropic (monofringent) - neutral fats & fatty acids
  - Anisotropic (birefringent) - any crystalline lipid
  - Maltese cross (birefringent) - cholesterol esters
- Also use phase contrast microscopy
Polariser and phase contrast
3. Reduction of osmium tetroxide

- Colourless $\text{OsO}_4$ to black $\text{OsO}_2$
- Marchi’s for degenerate myelin
- After normal myelin has been oxidised by chrome salts, it will not react with osmium tetroxide
- Degenerate myelin contains oleic acid which is not oxidised by chrome salts and will reduce osmium tetroxide to black osmium dioxide
4. Fat soluble dyes

- These dyes have a high affinity for fats, lipids, lipoproteins and triglycerides
- The fat soluble dyes are oil red O, Sudan II, Sudan III, Sudan IV and Sudan black B
- Staining solutions are generally alcoholic
Oil red O

- Demonstrates neutral lipids and fatty acids
- Oil red O is a fat-soluble dye
- More soluble in fat than in the dye solvent
- Solvent is usually isopropanol
- Cut frozen sections, usually leave unfixed
- Stain in oil red O and counterstain
- Mount in aqueous media
Oil red O
Sudan IV and Sudan black B
Bromine - Sudan black B

- One section stained with Sudan black after bromination
- Control section stained without bromination
- Cholesterol, cholesterol esters and phospholipids stain blue / black but remain unstained in control
5. Histochemical methods
Nile blue sulphate

- Demonstrates acidic and neutral fats
- Nile blue sulphate contains 2 components
- Red oxazine which dissolves in neutral fats
- Blue oxazine reacts with fatty acids & phospholipids
- Fix frozen sections in formol calcium for 1 hour
- Method requires 2 solutions:
  - Nile blue sulphate at 60°C for 30 minutes
  - Methyl green solution for 5 minutes
Nile blue sulphate

Neutral fats, oils and cholesterol esters - red
Fatty acids and phospholipids - blue
Perchloric acid-naphthoquinone (PAN)

- Cholesterol and cholesterol esters stain grey/blue
Other histochemical methods

- Luxol fast blue - phospholipids (and myelin)
- Baker’s acid haematein - phospholipids
- Fischler’s method - fatty acids
- Osmium tetroxide - a naphthylamine (OTAN) - phospholipids - useful for differentiating hydrophilic lipids (sphingomyelin, cerebrosides etc) from hydrophobic lipids (fatty acids & cholesterol esters)
- Fluorescent dyes such as phosphine 3R and benzpyrene
Why demonstrate fat

- Fat may appear abnormally as a result of trauma such as bone fractures
- Fat released into bloodstream can cause emboli
- Identify fatty tumours such as liposarcoma
- Identify fat in other pathological conditions
Lipid pathology

1. Fatty degeneration
2. Lipoma
3. Liposarcoma
4. Atheroma
5. Thecoma
6. Tay-Sach’s disease
1. Fatty degeneration

- Also known as steatosis or fatty change
- Abnormal retention of lipid within cells
2. Lipoma

- Benign tumour composed of adipose tissue (body fat)
- Most common benign soft tissue tumour
- Usually moveable and painless
3. Liposarcoma

- Rare, malignant tumour arising in fat cells deep in soft tissue such as the thigh
- Large and bulky often with satellites outside of the main tumour
3. Liposarcoma

- Undifferentiated liposarcoma left side
- Differentiated liposarcoma on right side
- Benign fatty tissue in the centre (has fewer blood vessels)
4. Atheroma

- Swelling in arterial walls with macrophages that contain fatty acids and cholesterol (clefts)
- Also contain calcium and fibrous connective tissue
- Occurs in atherosclerosis
5. Thecoma

- Benign ovarian tumour composed of theca cells
- Oestrogen producing
- Occurs in older women, generally after the menopause
- Tumour cells have abundant lipid-filled cytoplasm
6. Tay Sach’s disease

- Known as gangliosidosis or hexosaminidase A deficiency
- Caused by gangliosides accumulating in nerve cells
- Progressive mental deterioration in children with death usually by 4 years
- Lipid stored as concentric, laminated bodies