Autoradiography

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Principle

- **Ionising radiation acts upon a photographic emulsion or film to produce a latent image much as does visible light. This is called autoradiography.**
- The emulsion or film contains silver halide crystals. As energy from the radioactive material is dissipated the silver halide becomes negatively charged and is reduced to metallic silver, thus forming a particulate latent image.
- Photographic developers show these silver grains as a blackening of the film, then fixers are used to remove any remaining silver halide and a permanent image results.
- It is a very sensitive technique and has been used in a wide variety of biological experiments.
- A good example is autoradiography of nucleic acids separated by gel electrophoresis.

Suitable isotopes

- In general, weak β-emitting isotopes (e.g. 3H, 14C and 35S) are most suitable for autoradiography, particularly for cell and tissue localisation experiments.
- This is because the energy of the radiation is low.
- The sample must be close to the film, the radiation does not spread out very far and so a clear image results.
- Radiation with higher energy (e.g. 32P) give faster results but poorer resolution because the higher energy negatrons produce much longer track lengths, exposing a greater surface area of the film, and result in less discrete images.

Choice of emulsion and film

- Autoradiography emulsions are solutions of silver halide that can be made to set solid by the inclusion of materials such as gelatine.
- This can be used for example for autoradiography of microscope slides.
- X-ray film is the alternative and is used for gels.
- Films differ in sensitivity; advice on what to use is provided by the manufacturers.

Direct autoradiography

- In direct autoradiography, the X-ray film or emulsion is placed as close as possible to the sample and exposed at any convenient temperature.
- Quantitative images are produced until saturation is reached.
- The shades of grey in the image are related to a combination of levels of radiation and length of exposure until a black or nearly black image results.
- Isotopes with an energy of radiation equal to, or higher than, 14C (E_{max} = 0.156 MeV) are required.
- The higher the energy the quicker the results.

Fluorography

- If low-energy β-emitters are used it is possible to enhance the sensitivity several orders of magnitude by using fluorography.
- A fluor (e.g. PPO or sodium silicate) can be used to enhance the image.
- The β-particles emitted from the isotope will cause the fluor to become excited and emit light, which will react with the film.
- This has been used for example for detecting radioactive nucleic acids in gels.
- The fluor is infiltrated into the gel following electrophoresis; the gel is dried and then placed in contact with a preflashed film.

Intensifying screens

- Intensifying screens are used when obtaining a fast result is more important than high resolution.
- It is useful for example in gel electrophoresis or analysis of membrane filters where high-energy β-emitters (e.g.32P-labelled DNA) or γ-emitting isotopes (e.g. 125I-labelled protein) are used.
- The intensifying screen consists of a solid phosphor, and it is placed on the other side of the film from the sample.
- High-energy radiation passes through the film, causes the phosphor to fluoresce and emit light, which in turn superimposes its image on the film.
- The reduction in resolution is due to the spread of light emanating from the screen.
- When intensifying screens or fluorography are used the exposure should be done at low temperature.

Preflashing

- The response of a photographic emulsion to radiation is not linear and usually involves a slow initial phase (lag) followed by a linear phase.
- Sensitivity of films may be increased by preflashing.
- This involves a millisecond light flash prior to the sample being brought into juxtaposition with the film and is often used where high sensitivity is required or if results are to be quantified.

Quantification

- Autoradiography is usually used to locate rather than to quantify radioactivity.
- However, it is possible to obtain quantitative data directly from autoradiographs by using digital image analysis.
- Quantification is not reliable at low or high levels of exposure because of the lag phase or saturation, respectively.
- Preflashing combined with fluorography or intensifying screens create the best conditions for quantitative working.