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Overview of the NatSCA Biochemistry Seminar
Simon Moore, Course Coordinator

Kew Gardens Jodrell Laboratory
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The theme of the day largely concerned the biochemical make-up of our specimens and what can happen to them, at a molecular level, under certain conditions.

The first two talks concerned a brush-up of biochemistry and terminology for those of use who were less-experienced or who had not partaken since 'A' level or University days!
Simon Moore introduced the seminar to some basic facts about organic molecular structure, bonding, cyclic structuring and amino acids which led directly into a more detailed continuation by David Lampard (Dundee) who led us further through the structures of proteins, including secondary and tertiary 'foldings', sugars and other carbohydrates, enzymes and all the other relevant building blocks of biochemistry.

Following a quick break to refresh our already-boggling brain cells, Amandine Pequignot (Paris NHM) outlined the molecular processes that occur with fluid preservation. This naturally included the fluid fixation process that prevents protein breakdown into component amino acids by cross-linking them and coagulating cell contents but still preserving the methylene –CH2 bridges that bond proteins together. We should always use formalin or formalin-based fixatives and then transfer specimens to alcohol/s if required. IMS is really useless as a fixative since it simply displaces –OH ions in proteins and reduces inter-molecular spaces leading to stiffness. On the other hand if used as a storage medium, it will slow down or prevent harmful hydrolysis. She mentioned the differences between multi-layer water within proteins which is weakly bonded by -H- bonding and van der Waal's bonds, as opposed to bond water which, if removed, reduces the gaps between molecular layers and leads to specimen rigidity.

She also re-used some of her talk from the NatSCA/SPNHC conference (2005) about comparing the tensile strength of mammal skins fixed in various fluids including propan 2-ol (isopropyl alcohol), concluding that we should be using 5% formalin (2% formaldehyde) and that the normal fixation strength can lead to too much rigidity in (mammal) specimens. Another interesting conclusion related to rehydration which was found to be easier from alcohol pseudo-fixation than from formalin fixation.

Carrying on this theme of fixation, Jules Carter then treated us to the latest findings in DNA fluid preservation. He disfavoured the use of Steedman's 1979 fixative as suitable for larger specimens – it was after all, devised for zooplankton – although this author still finds it useful for certain macro-invertebrates. Concerning the continuing debate about formalin scrambling or interfering with DNA, he informed us that formalin-fixed DNA is perfectly OK but would require extraction through critical point drying. Alcohol fixation may release denaturants leading to breakdown of DNA structure. Many specimens exude certain compounds during fixation, extracted by the fixative and although these may colour or appear to contaminate the fluid they will reach an equilibrium. Changing the fixing or preserving fluid will upset this equilibrium and could bring about further extractions from tissues but that certain tannins and resins, extracted during plant fixation, and which also contaminate the fluid, can intefere with plant DNA and would therefore need to be removed. Ethyl acetate, used as a killing vapour for entomological specimens, is disastrous for DNA extraction – the reason is presently unknown.

He also added to the alcohol fixation problem since IMS and ethanol will denature and aggregate proteins and cause disruption to tertiary hydrophobic bonding which results in steric arrangement and produces isomers (mirror image molecular structures) which may have entirely different properties. Dilution of alcohols will initiate further hydrolysis due to added amounts of water which is bad for specimen tissue stability.
In conclusion, to preserve DNA effectively:
Cryo- or low temperature freezers maintaining tissues at -80°C, or storage in liquid nitrogen but requires expensive and dedicated equipment.

Drying – Critical Point Drying using acetone or absolute ethanol, or freeze-drying followed by storage in a cool and dry environment.

Finally: rehydration removes vital chemical elements through detergency action and must be only be carried out for gross anatomy tissues and specimens, otherwise leave the tissue in a dry state.

Some may remember Bob Stoddart from Velson Horie's Manchester Museum fluid preservation seminar in 1989. He gave 3 presentations showing the basic structure and compounds involved in biological museum specimens and how, under changing conditions, these vital compounds can become altered, whether by breakage or shortening of a molecular chain at some vital and 'vulnerable' point, and how other compounds (some deteriorative) might be formed as by-products. These facts he linked to DNA structure and to medical and histological radical facts and it made me wonder how on earth we have managed to steer our collections (hopefully) through all these biological minefields over time!

His first talk comprised deterioration of plant specimens in collections, at first outlining the importance of cell wall structure, reminding us of the primary layer comprising rhamnose and galacturonic acid, a secondary matrix of a different mix of carbohydrates and, for woody stems, a tertiary and impermeable layer of lignin.

He also reminded us about the composition of cellulose as a β-1,4, glucan except that it contained the carbohydrate mannose giving rise to gluco-mannan fibrils, also the composition and relevance of pectins, some of whose structures are so huge that they have not yet been 'mapped'! He explained about the physical versatility of carbohydrate complexes – the toughness of ivory nuts containing a rigid form of gluco-mannan against a similar structure in the root slime of a certain lily which has the opposite physical properties. He continued in similar vein outlining the components of other plant structures in our care and then switched to the effect that herbarium drying had on these components. How the removal of structural water causes woody structures to crack along medullary rays but that less lignified tissues can cope with this effect without breakdown. Fluid-preservation can be fine except that water exchange between cells and the preservative can occur and that complex molecules can expand into large gelatinous deposits. Lipids are extracted in IMS and lipocyte membranes can become degraded but are stable in dry form. Nonetheless, alcohol preserves gross tissues quite well except for algae which live in water and therefore require a more aqueous preservative.

He concluded with another word of caution relating to DNA, that it is vulnerable to photo dimerism in sunlight but that a glass barrier of more than 257 nm will be sufficient to absorb any harmful effect.

Adrian Doyle, ably assisted by Karen Roux from Thermolignum outlined the benefits of the Thermolignum process and how it compares favourably with other pest eradication processes by maintaining RH (within a 10% total-fluctuation parameter) and other environmental-relevant equilibria and guarantees a 100% kill over 24 hours. Invasive pests of museum objects are killed by 3 hours at 55°C as opposed to -30°C for 72 hours. Polymers can become 'unlaced' by freezing or non-equilibrated heating through their thermoplasticity properties. DNA is affected by exposure to naphthalene but is unaffected by the Thermolignum process. There were some warnings about the Thermolignum process. Phthalate-based adhesives can become yellowed by the process. A tight rebate on a taxidermy case can cause (old) glass to crack and may require a slower temperature increase and decrease to prevent this.

He concluded with a warning about the cumulative effect of varying pesticides and how these can affect specimen stability (Vicky Purewal is researching this presently).

Bob Stoddart then warned us about the effects that omnipresent airborne fungal spores can have on human physiology. He explained how hospital patients could mysteriously deteriorate and die due to cryptic fungal infections, he continued by showing fungal temporary surface infections as opposed to invasive - fungal hyphae actually spreading through tissues - demonstrated at a histological level using Gridley's Feulgen reaction or Grocott's silver impregnation. He also warned of the possible grave consequences of inhaling particles from dried pigeon droppings or receiving mammals specimens from California which might carry *Coccidioides* sporangia. After this gloom and doom which had us thoroughly washing our hands before the next tea break, he outlined how fungi produce digestive hydrolases and that some, such as *Aspergillus niger* contains a cocktail of 6 enzymes that can cause rapid tissue breakdown.

For his final presentation, Bob talked about pigments and how they can break down in museum collections through various agents. He started by explaining some light / colour physics and how iridescence and interference colours are produced through micro-ridged or micro-layered surfaces, e.g. butterfly wing scales. He

went on to explain how pigments are molecularly structured, linked through a single and double-bond alternating linkage or chromophore and how this can be shortened by photonic weakening of one of the double-bonded linkages and causing a reduction in colouration (eg. fading) and how a lengthening of the chromophore causes darkening. He mentioned the carotenoids as examples and how the various forms of carotene (α- β- γ- δ- &c) had slightly longer or shorter chomophores and therefore showed different colours. He showed how the blood pigmenting agent Haem – an iron-based tetrapyrrole (with carboxyl groups) can change its composition out of the bloodstream as it loses the iron ion and changes to biliverdin and bilirubin which explains how bruises change colour. He also showed how the magnesium tetrapyrrole, more familiar as chlorophyll, can similarly lose its magnesium ion to change to phaeophytin A and B becoming brown which is why marine brown algae, need only a small amount of sunlight to photosythesize, hence the kelp niche largely in the lowest intertidal zone. Another example is of porphyrins (found in deep sea jellyfish) which are dissolved by alkalis by removing the iron ion, just as acidified acetone removes haem from haemoglobin. He then ventured into anthocyanins and the families of phenolic –OH (hydroxyl) groups that react with amines and iron, especially ferric iron, to produce very strong pigments but how phenolics are prone to fading through oxidation. He told us how a strongly-pigmented flower can change from bright pink to green or blue when a burning cigarette, combining heat and tobacco smoke, is held very close. He also mentioned the litmus test using anthocyanins and how the red oxonium and the blue (basic) quinone colour reactions are reversible.

He then talked about xanthopterin in insects (with other pterins) and how these too can change colour through oxidation, also how lignin will oxidise in sunlight causing furniture to fade. Fungi contain polyalkynes with triple-bonded carbon atoms but that these triple bonds are amazingly weak and can easily break causing fading, something I have found with some, but not all, of the freeze-dried Hampshire fungal collection.

Papers Given at the Biochemistry Seminar, Jodrell Lab, Kew 9.11.2006

Basic Biochemicals
- David Lampard

Biochemistry is the branch of organic chemistry that deals with the molecules involved in biological processes and structures. Before describing some of the main groups of biochemicals it is worth defining some of the terms most frequently used when describing biochemicals and their reactions

There are three types of chemical bond that connect atoms and molecules together;

1. **Covalent Bond**, formed by the sharing of one or more electrons, especially pairs of electrons, between atoms.
2. **Ionic Bond**, formed by two ions with opposite charges, characteristic of salts. Also called *electrovalent bond*.
3. **Hydrogen Bond**, a hydrogen atom of one molecule is attracted to an electronegative atom, especially a nitrogen, oxygen, or fluorine atom, usually of another molecule.

Covalent bonds between carbon atoms help form the long chains of biochemicals.
Hydrogen bonds are often involved in determining the shape of large molecules

Displaying formulae

A molecular formula shows the relative abundance of the atoms more electrons, especially pairs of elec-