electrical properties of the films is an essential link in any comprehensive investigation of film growth. Carpenter and Wilcock (both of whom are working under the direction of Mr. D. Campbell, one in a university, the other in a commercial laboratory) are using very sensitive measuring equipment for the study of initial stresses in thin films of a type on which structural data are available. They are at present seeking to relate the onset of visible nucleation with the first appearance of stress.

White has investigated the preparation of thin insulating films by 'field-assisted anodization' or 'biased sputtering'. Results so far do not fully confirm claims made by some workers in the United States that the process is comparable with aqueous anodization. It appears to differ not only in its low-current efficiency but also in the properties of the films prepared, and the independence of their thickness of applied bias voltage.

J. R. Balmer
J. H. Bruce

MECHANISM OF CELLULASE ACTION

A ONE-DAY symposium on the mechanism of cellulase action, under the auspices of the Molecular Enzymology Group of the Biochemical Society, was organized by the Shirley Institute on May 28, 1965. Dr. J. Honeyman, who presided, commented that Mr. Selby took this opportunity to arrange a symposium because both Dr. Elwyn T. Reese, from Natick, Massachusetts, and Dr. Bruce A. Stone, from the University of Melbourne, were at present working in the United Kingdom. The Shirley Institute had for several years been active in cellulase research, particularly in work designed to elucidate the mechanism of the biodeterioration of cotton.

K. Selby (Shirley Institute) gave the first paper on the enzymatic degradation of cotton. He stressed that since many 'cellulases' classified by Reese as C_x can attack soluble cellulose derivatives or swollen cellulose but not native celluloses, the structure of the solid cellulose must govern its susceptibility to attack; if we knew more about the structure we would understand the enzymes better, and vice versa. Although opinion is now tending towards the belief that cotton is entirely composed of crystalline microfibrils without amorphous regions, there exist inhomogeneities in the structure, ranging in size from interfibrillar holes to the layer-structure of the cotton hair, and these may provide sites for enzymatic attack. If cotton could be chemically modified in these accessible regions it might be protected against attack, and this has been done with some success by Mr. Colbran at the Institute by reaction with phenyl isocyanate under non-swelling conditions. Work on the cellulase system of *Myrothecium verrucaria* was reported; exclusion chromatography separated a C_x from two other components both capable of weakening cotton, and both, like the activity of the crude filtrate, 'exhausted' in the process. The cellulase of *Trichoderma viride* does not suffer from this disability and seems to be a better agent for examining cellulose structure.

In subsequent discussion, J. O. Warwicker (Shirley Institute) made it clear that it is still possible to explain the physical and chemical properties of cotton which had previously been attributed to the amorphous part of its structure.

B. A. Stone (University of Melbourne) then gave an account of his work on the purification of the exo-β-1,4-glucanase (cellulase) of *Aspergillus niger* and its action on glucans with mixed 1,4- and 1,3-linkages. Elution chromatography removed cellobiase (probably an exo-β-1,4-glucanase) and endo-β-1,3-glucanase (laminarinase) and the resulting cellulase digested carboxymethylcellulose with a fall in viscosity that demonstrated its chain-cleaving action. Barley glucan was broken down by this cellulase to give some glucose, cellobiose and mixed-link tri- and tetra-saccharides both with 1,4-linkages at the reducing ends. This result confirms that of Perlin and Reese with *Streptomyces* cellulase and should be interpreted in the same way. However, Stone found that the cellulase still contained exo-β-1,3-glucanase and that after removing this by adsorption on insoluble laminarin the products of digestion of barley glucan were significantly changed. Glucose was practically absent, but there were present, as stable products, about 20 per cent of pentasaccharides and higher sugars which, so far as their structures had been determined, all showed the cellobiose grouping at the reducing end. This purified cellulase should be a powerful tool in the investigation of mixed-link glucans.

E. T. Reese (Quartermaster Research and Engineering Center, Natick, Massachusetts) reviewed work on β-glucanases, leaving a vivid impression of the multitude and variety of these enzymes and the complexity of their specificity. In particular, the work of Perlin showed that several endo-glucanases are not specific to the bond being broken but to the nature of the reducing end-unit being liberated. A surprising example of this is that, acting on a glucan with alternate 1,3- and 1,4-linkages, cellulase, producing cellobiose, would attack the 1,3-linkages, and laminarinase, producing laminaribiose, would attack the 1,4-linkages. How far this is true of all endo-glucanases is not clear. The growing class of known exo-glucanases act from the non-reducing end of the glucan; many of them liberate disaccharide molecules, and some have been shown to attack more than one kind of bond. Some exo-glucanases are blocked in their endwise action by substituent groups and branches in the glucan, but a case was given of an exo-1,3-glucanase, from a basidiomycete, which is not stopped by 1,6-branchpoints in a 1,3-glucan.

C. C. Maitland (Shirley Institute) described present work on the cellulase of *Trichoderma viride* and its attack on cotton, which it is able to solubilize entirely. Exclusion chromatography on 'Sephadex' *G*-75 separates the system into three major components. One component, of low molecular weight, accounts for the greater part of the activity against carboxymethylcellulose but plays no recognizable part in the solubilization of cotton. The two other components are probably identifiable with Reese's C_x and C_1, the C_x having activity against carboxymethylcellulose and cellobiose, while C_1 has little or no such activity but acts synergistically with C_x in solubilizing cotton. The nature of this synergism, first found by Reese and co-workers but now shown more markedly than before, was discussed. The implied division of labour could be based on the structure of the cotton hair, but this seems unlikely because acid degradation and ballmilling, although destroying the large-scale structure of cotton, do not entirely remove the synergistic effect. It is generally assumed that C_1 initiates the attack on cotton and C_x follows; this is very likely, but there is no direct evidence for it. However, the small solubilizing power of C_1 acting alone is enhanced when the incubation with cotton takes place on a dialysis membrane; this indicates that an inhibitory product of C_1 action, which C_x would presumably have removed, is now able to diffuse away.

G. Halliwell (University of Strathclyde) then spoke of his work on cellulases at the Rowett Institute. Rumen bacteria, although capable of extensive breakdown of solid cellulose, do not yield powerfully cellulolytic filtrates, but *Trichoderma* species do both, and the breakdown of fibrous cellulose (cotton) was investigated by using their filtrates. Of particular interest was the observation that an early stage of attack, noticeable by the breakdown of the cotton into insoluble fragments, of which the smallest were capable of passing through a

No. 3 porosity glass sinter, was distinct from the subsequent conversion into reducing sugars. This initial attack was most rapid at a markedly lower *p*H and might perhaps be caused by a different enzyme.

N. J. King (Forest Products Research Laboratory) described the present state of his work on the cellulase of *Coniophora cerebella*. This is classified as a brown-rot fungus, capable of digesting the polysaccharide of wood and leaving a skeleton structure of lignin. Electron-micrographs showed how this attack was widespread and not confined to the immediate neighbourhood of the fungal hyphae. The widespread nature of the attack was also illustrated by the production, in the early stages of fungal invasion, of more soluble sugars than the fungus can utilize. *Coniophora* has been found to grow easily on cellulose in submerged culture, but cellulase active against compact native cellulose has not yet been obtained. Filtrates contain a cellulase active against swollen cellulose, carboxymethylcellulose, and against cellodextrins, particularly cellotetraose and higher analogues. This cellulase has been successfully fractionated by elution chromatography on DEAE-cellulose giving three components active against carboxymethylcellulose, which were shown to be distinct by electrophoresis on polyacrylamide gel.

In a general discussion, C. van Bochove (T.N.O., Delft, Netherlands) mentioned his experience of an interesting case of protection of cotton from microbial decay by substitution reaction at the right sites. Methylolchloroacetamide reacts with cotton and protects it against decay at a degree of substitution of 0·06. If the chlorine is removed by hydrolysis, protection remains; yet the resulting substituent group, applied directly as methylolglycolamide, gives no protection, presumably because the substitution is not in the right place. J. Dlugosz (Shirley Institute) spoke of the interesting surface features shown by electron-microscopy of cotton attacked by the cellulase of *T. viride*.

C. C. MAITLAND

BINDING OF XENON TO SPERM WHALE MYOGLOBIN

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IN 1946 Lawrence *et al.*[1] and Tobias *et al.*[2] suggested that xenon might be expected to have anaesthetic effects in consequence of its relatively large inducible dipole. In 1951 Cullen and Gross[3] confirmed that xenon was indeed an anaesthetic agent in man[4]. The electron cloud of xenon is, of course, spherically symmetrical, so its narcotic activity cannot depend on any specific structural grouping as had often been supposed[5], and therefore novel theories of anaesthesia were proposed to explain the action of anaesthetics in general and of xenon in particular[6–8].

During further investigations of the anaesthetic action and distribution of xenon it became apparent that more xenon is transported by the blood than would be expected from its solubility in the plasma. Solubility experiments then proved that xenon reversibly binds to haemoglobin and myoglobin[9–11]. Since the structures of sperm whale met- and reduced myoglobin have been determined, and nearly all the 1,260 non-hydrogen atoms have been located (J. C. Kendrew and H. C. Watson, to be published), it seemed possible to determine the mode of attachment of xenon to myoglobin without difficulties by the difference Fourier method. Such an X-ray diffraction analysis of crystalline sperm whale myoglobin in equilibrium with xenon has now been carried out. The results prove xenon to be bound to one specific site which is buried in the interior of the molecule and is nearly equidistant from one of the pyrrole rings in the haem group and the ring of the haem-linked histidine. The experimental details and results of the investigation follow.

Sperm whale metmyoglobin, prepared by the method of Parrish and Kendrew[12], was crystallized from an 80 per cent ammonium sulphate solution at *p*H 6·8. Crystals were mounted in a special cassette and equilibrated with xenon at 2·5 atm. for 12 h before and during their X-ray exposure. The intensities of the *hk*0, *h*0*l* and 0*kl* reflexions were collected to 2·8 Å resolution on multiple film precession photographs with copper $K\alpha$ radiation from a rotating anode X-ray tube. The exposure times were much longer than usual owing to the strong absorption of the X-rays by the xenon gas in the cassette. The intensities were measured with a semi-automatic microdensitometer; corrected for Lorentz, polarization and temperature factors and then scaled to the native metmyoglobin data.

Difference Fourier projections were calculated using appropriate versions of the general equation:

$$\Delta\rho_{(x,y,z)} = \frac{1}{V}\sum_h\sum_k\sum_l\{|F_{(xe,p)}| - |F_p|\} \exp\{-2\pi i(hx + ky + lz) + i\alpha\}$$

$|F_p|$ and $|F_{xe,p}|$ represent the moduli of the structure amplitudes of the native myoglobin and its xenon derivative respectively, $\Delta\rho_{(x,y,z)}$ the difference in electron density between the two compounds. The phase angles used were those for native sperm whale myoglobin, as determined by multiple isomorphous replacement[13].

The use of a set of only approximate phases in a difference Fourier synthesis results in reduced peak heights for non-centrosymmetric projections. Luzzati[14] showed that the peak height of an atom not included in the phase determination depends on the ratio of the excluded electrons to the included ones. In this case, where the excluded electron density is small, the theoretical expected reduction of peak heights in non-centrosymmetric sections is of the order of 40 per cent and agrees well with the observed results.

Each of the difference electron density maps of the *hk*0, *h*0*l* and 0*kl* projections (Fig. 1) shows only one, nearly circular, peak corresponding to a spherical atom with coordinates $x = 0{\cdot}177, y = 0{\cdot}864, z = 0{\cdot}168$. The remaining areas of the maps are relatively featureless, indicating that the degree of isomorphism is high and that xenon atoms are not present at subsidiary sites to any appreciable degree.

The xenon atom is nearly equidistant from the haem-linked histidine and a pyrrole ring of the haem group; it is in contact with all the atoms in both rings. The neighbourhood of this binding site is shown in Fig. 2*a* and *b*, and the relevant approach distances are tabulated in Table 1.

The xenon atom in the myoglobin interior lies between a non-polar area and an area which is partially polar, indeed charged. This suggests that this complex is stabilized by charge-induced dipole moments, by dipole-induced dipole moments and by London interactions. In addition, there is an entropy gain due to the transfer of the xenon from the aqueous surface into the interior of the protein. Unfortunately the charge distribution on the