deterioration caused by macro-organisms, and it is questionable whether this fairly reflects the economic importance of this type of bio-deterioration. Studies at present of macro and micro bio-deterioration seem to share a common approach only in the field of biocides. It is to be hoped that this difficulty will be resolved in time for the second international symposium.

PHOTOMOLECULES

New Vision

from our Molecular Biology Correspondent

PRESENT knowledge of the chemistry of vision derives almost entirely from the work of Wald and his group, who during the past fifteen or so years have delineated the steps involved in the bleaching and regeneration of the visual pigment. Wald's Nobel lecture (see *Nature*, **219**, 800; 1968) makes compelling reading. One avenue which this work has opened leads to the conformational behaviour of the rhodopsin molecule which, as Wald showed, displays chemical differences when the prosthetic group—the 11-*cis* retinal chromophore—is photoisomerized to the all-*trans* form and in due course detached, leaving the apoprotein opsin. Several workers have now shown that a change in optical activity accompanies this process, and a further interesting study, along complementary lines, has now been published by Heller.

In the first of two papers (*Biochemistry*, **7**, 2906; 1968), Heller describes a procedure for making the disaggregated native pigment soluble. This has been a problem, because the protein exists naturally in a non-aqueous membrane environment and is unusually non-polar in character and insoluble in water. While anionic detergents (dodecyl sulphate) cause denaturation, Heller finds that a cationic detergent, cetyltrimethylammonium bromide, disrupts the pigment aggregates but leaves the protein in its native state, at least so far as can be judged by its unimpaired visible absorption spectrum (bleaching is always the first consequence of denaturation). The protein, which carries bound detergent, is essentially monomeric and can now be subjected to further purification by gel filtration (all, of course, in the dark). The product is homogeneous on the columns and in polyacrylamide gel electrophoresis.

Amino-acid analysis confirms the unusually low content of charged residues. It also emerges that the rhodopsin is a glycoprotein, containing altogether some 4 per cent of carbohydrate—glucosamine, mannose and galactose. The solubility of the preparation makes possible an estimate of molecular weight by means of the elution volume on a gel filtration column calibrated with a series of standard proteins. This method is at best approximate, and is here complicated by the necessary assumption that the cationic detergent, which must be present, will be bound by all proteins to proportionately the same extent. The procedure is, however, good enough to demonstrate that the molecular weight has the minimum value consistent with the composition—that is to say, it corresponds to one chromophore per molecule and is about 27,000.

In a second paper (*ibid.*, 2914), Heller examines the effect of bleaching on the protein. Wald and Brown

showed in 1952 that exposure to light is followed by the emergence of available cysteine residues, and a conformational change in the protein has also been demonstrated. Heller has used a colour reaction (DTNB thiol reagent) to measure the concentration of new thiol groups after bleaching, and finds one per protein molecule. As a direct indication of a conformational change, he finds a small but very reproducible shift in the elution position on a gel filtration column. This is in the direction of an increase in hydrodynamic radius, and is unchanged when the loss of the isomerized chromophore from the protein is prevented by reduction of its Schiff base link to a lysine side chain. A mixture of bleached and unbleached material elutes clearly as two components. This parallels a situation encountered in several enzymes possessing prosthetic groups: the apoenzyme exists in a less compact, probably less ordered and certainly inactive state, more sensitive to denaturation and proteolysis, sometimes apparently containing less α -helix. This condition is reversed on addition of the cofactor.

ENZYMES

Thiol Groups Everywhere

from an Enzymologist

THIOL groups are the standby of many speculative enzyme mechanisms, and they have cropped up recently in contexts as disparate as ribosomes and nerve receptor protein. As the most potent nucleophile in the cell, this is perhaps inevitable, but in fact there has been little rigorous exploration of the behaviour of the thiol group when embedded in a macromolecule except for the frequent observation that individual enzyme thiols often react faster—or slower—than they should.

An attractive model system is described by P. Heitmann in the latest issue of the *European Journal of Biochemistry* (**5**, 3; 1968). He measured the S_N2 reactivity of aqueous solutions of *N*-dodecanoyl-DL-cysteinate with various uncharged reagents. This long fatty thiol compound forms anionic micelles in water—a fair model of globular protein in that a hydrophobic core is sheathed by a layer of ionic groups. Dr Heitmann has found that the thiol groups of the micelle have very low reactivity, but that when the micelle is made cationic by the addition of hexadecyltrimethylammonium bromide, the thiols become hyper-reactive; with iodoacetamide, they are 60–100 times more reactive than a simple thiol group. The results are a powerful demonstration of the importance of specific microenvironment in thiol reactivity. Dr Heitmann proposes that in the transition state, juxtaposed positive charges assist the departure of the negative group in the cationic micelle, and that the reverse happens in the anionic micelle.

The theme of thiol micro-environment is continued in a paper appearing in the *European Journal's* young stablemate *Febs Letters* (**1**, 25; 1968). Dr Eisele and Dr Wallenfels there describe an ingenious way of using thiol reactivity to probe protein conformation change. Previous papers from Dr Wallenfels' laboratory have established that hydrogenases with A-stereospecificity with respect to their coenzyme react faster with D-iodopropionic acid than with its L antipode, and B-specific dehydrogenases do the reverse. The new paper shows that yeast glyceraldehyde-3-phosphate dehydro-