now determined the initial site of virus synthesis in such heterokaryons. Assaying for SV40 virus, Wever *et al.* found it first in the nucleus from the transformed cell and only later in the nucleus of the permissive cell. The first event in rescue appears therefore to be the activation of the SV40 genome in the transformed cell nucleus and not the transfer of SV40 DNA to the permissive cell nucleus.

PROTEINS

Rubredoxin Revealed

from our Molecular Biology Correspondent

Now that so many principles of globular protein structure have been established by X-ray analysis, crystallographers are increasingly (possibly even for reasons of *Lebensraum*) lifting their gaze from the familiar hydrolytic enzymes and haem proteins to size up various altogether more esoteric species. There are indeed many interesting problems to be solved; metalloproteins, for example, have both irked and intrigued inorganic chemists for some time, because many of their properties—the absorption spectra for instance—are often anomalous, and have no counterpart in their accustomed scheme of things. It is thought that rigid constellations of binding groups in the protein permit the formation of highly strained metal complexes, which are associated with such features as abnormally large extinction coefficients.

An interesting set of metalloproteins, which occur in plants and bacteria, are the non-haem iron proteins, such as the ferredoxins and rubredoxins. Both function as electron transport agents, though differing considerably in redox potential, and both have been the subject of intense speculation, centring on the nature of the protein–iron linkage. It has been concluded that the iron is in both cases bound to sulphur, but in the ferredoxins the sulphur is extrinsic to the protein (inorganic sulphur), whereas in rubredoxin it is present as cysteine. One laboratory embarked some years ago on the crystallography of a ferredoxin, another on a rubredoxin, and the second has now surfaced with an atomic resolution structure (Herriott, Sieker, Jensen and Lovenberg, *J. Mol. Biol.*, **50**, 391; 1970). This is no mean achievement, for the rubredoxin is one of the smallest globular proteins, with a molecular weight of 6,000. Proteins of this size present particular technical difficulties to the crystallographer, and the epic struggle to uncover the structure of insulin (also 6,000) is remembered with awe. When it is considered that the sequence of the rubredoxin is also not known, one is left with the feeling that Jensen and his group were leading with the jaw.

Nevertheless, with the aid of two isomorphous derivatives, they have solved the structure of clostridial rubredoxin to 2·5 Å. The molecule is roughly spherical, with a radius of some 12 Å. The polypeptide backbone can be traced, and reveals two sections of antiparallel β-structure, one a hairpin loop, the other formed by association of the N- and C-terminal segments. There is no α-helical structure at all—a unique situation amongst the proteins of known structure to date, and of some interest in regard to the notion, which has been propagated of late, that it is the α-helical regions, no matter how short, that nucleate the correct folding of the chain.

Despite the absence of sequence data, a number of the more prominent side chains can be identified, in particular the seven aromatic residues, all but one of which are internal. Four of the five prolines are provisionally assigned, and the long polar side chains can be made out, extending from the surface. The most cogent question to be answered, however, concerns the environment of the single iron atom. On the basis of earlier chemical evidence on the rubredoxin of another species, it has been suggested that the ligands are the four thiol groups of the protein, and this surmise is triumphantly borne out by examination of the structure. Three cysteines are resolved, and the fourth nearly so. Bond lengths and angles can be approximately measured, and the grand conclusion is that the iron complex is tetrahedral, or at any rate very nearly so. Inorganic chemists will no doubt be equal to the challenge of explaining the properties of the metal in terms of this structure.

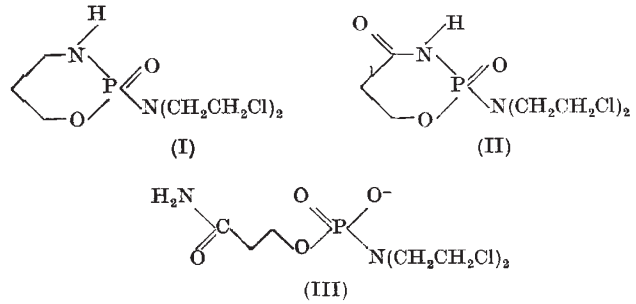
CANCER CHEMOTHERAPY

Drug Oxidation

from our Biological Chemistry Correspondent

CYCLOPHOSPHAMIDE (I) is widely used in the treatment of cancer, as, for instance, in the chemotherapy of leukaemia. It has been recognized that this drug appears to have no cytotoxic activity by itself but is transformed into an active alkylating agent by an oxidative process in the liver. Paradoxically, the activating enzyme system responsible appears to be a non-specific, mixed oxidase system which may also be involved in the activation of carcinogens as well as in the metabolism of drugs and steroids.

A group of chemists working at the Kettering–Meyer Laboratory in Birmingham, Alabama, has investigated the derivatives of cyclophosphamide found in the urine of dogs which were dosed with (I) by intravenous injection. Two major metabolites were detected of which one was purified and crystallized. Its spectroscopic properties suggest that it has the amide structure (II), which was confirmed by its reactions and by an unambiguous synthesis of the 4-ketocyclophosphamide. The other component of the urinary metabolites is likely to be the ring-opened product (III).



This finding confirms previous hypotheses that activation of cyclophosphamide involves oxidation at C_4. It also raises the question of the tautomeric behaviour of (II) which could influence the nature of its hydrolysis and therapeutic reactivity. It appears probable that 4-ketocyclophosphamide is either the active form of cyclophosphamide or a precursor of it.