NAVAL RESEARCH

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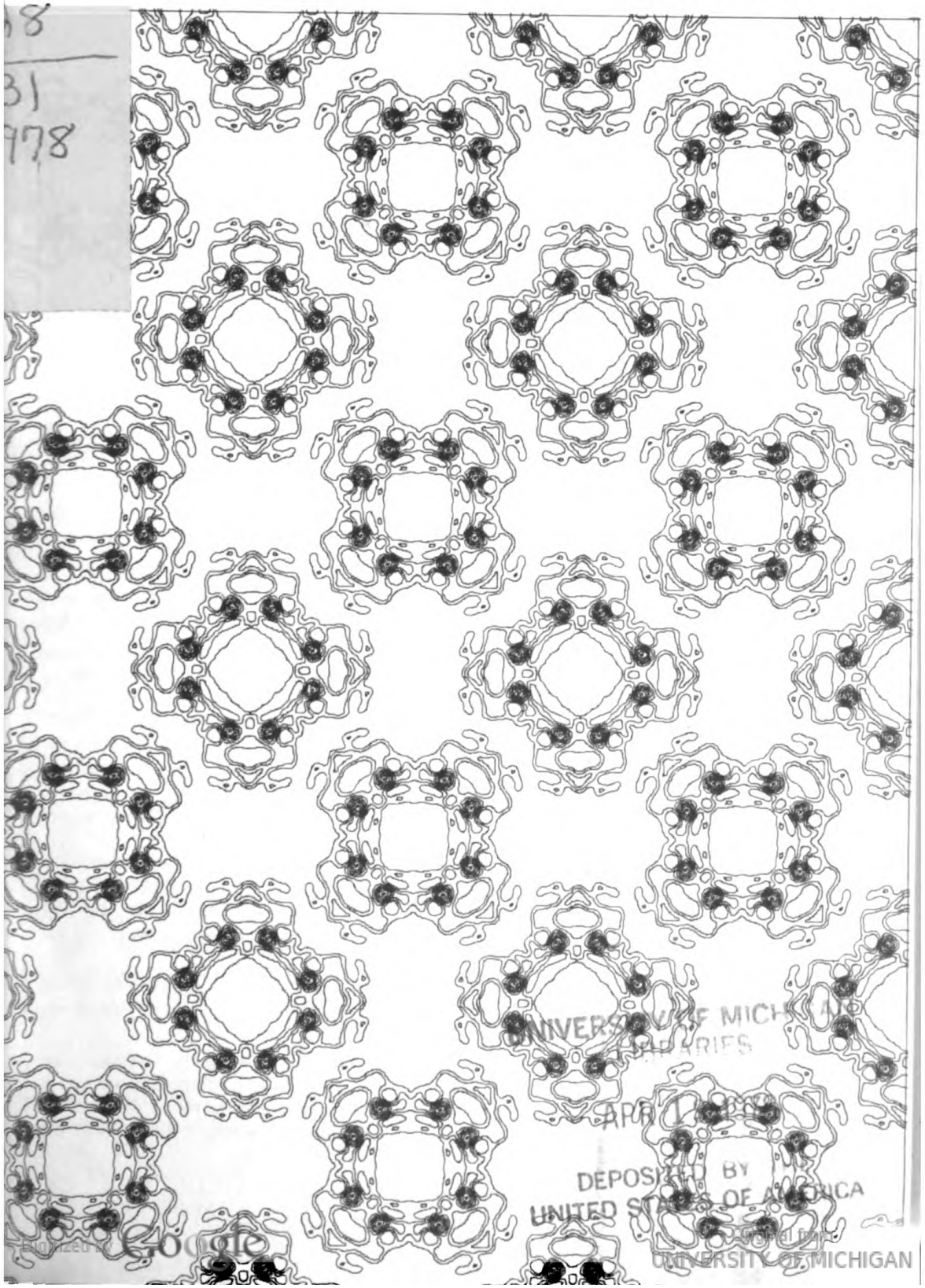

PROFILES IN SCIENCE

Dr. Daniel H. Wagner

Dr. Daniel H. Wagner is president of the operations research consulting firm, Daniel H. Wagner, Associates, Paoli, PA, which has been an ONR contractor since its founding in 1963. Dr. Wagner's firm has been the acknowledged leader in mathematical analysis of anti-submarine warfare (ASW), particularly in search problems. Its extensive ONR-sponsored work includes basic research in theory of search and detection and related probability theory, as well as Fleet field assignments under the ONR/Op-095 Tactical Development and Evaluation Program. This combination has resulted in computer assisted search programs which have directly benefited the Navy. The firm's search work originated with on-the-scene participation in the 1966 H-bomb search and the 1968 SCORPION search.

The early ASW orientation of his firm's work derived from Dr. Wagner's leadership of an ONR-sponsored study group on mass-produced ASW submarines. In 1959-65 he did considerable work in tactical applications on noise-speed trade-offs in ASW, built on the concepts of "acoustic advantage," "kinematic enhancement," and "secure sweep width," which he originally formalized and named.

While in the Navy's Operations Evaluation Group 1951-56, Dr. Wagner contributed to carrier warfare aspects of tactical development, Korean operations, and operational requirements, mostly on field assignments.

Dr. Wagner, who earned his Ph.D. in mathematics at Brown University, has published nine research papers on optimization, groups, and reliability and three expository papers, including a recent survey of measurable selection theorems, partly sponsored by ONR. Currently, he is on the National Research Council's Committee on Training in Applied Mathematics.

Biomolecular Transport of Oxygen — The Essence of Animal Life

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Introduction

Molecular oxygen is vitally important to animal existence. Man, for example, dies within a few minutes when deprived of oxygen. Yet it is not necessary for all life. Plants do not require oxygen; indeed they generate it. Many microorganisms (such as those that ferment grapes into wine) thrive in anaerobic conditions and some can live only in the absence of oxygen. What then is it that makes a continuous and plentiful supply of oxygen so uniquely indispensible to animal life? The answer lies in the very features that distinguish animals from other life forms, namely mobility and nervous activity. These processes consume great quantities of energy. It is molecular oxygen that makes the metabolic extraction of chemical energy from foodstuffs efficient enough to meet the energy demands of animal life.

In order to satisfy the great metabolic requirement for oxygen, most animals have developed a circulating body fluid that transports oxygen from the external environment to the tissues where it is needed. However, a circulatory system does not by itself solve the delivery problem; the solubility of oxygen in water is much too low to support respiration in active animals. Thus, nearly all bloods contain rich supplies of oxygen-carrying protein molecules to enhance the capacity for oxygen. The muscles of many animals also contain proteins that reversibly bind oxygen. These molecules facilitate the diffusion of oxygen through the muscle and can also provide a reservoir for the storage of oxygen.

This article reviews the importance of oxygen in metabolism and describes the diverse molecular devices that nature has evolved for its transport. The need for oxygen transport is laid out in a concise primer on biochemistry. The biological distribution and molecular architecture of the several different oxygen-carrying proteins are described in some detail. Particular attention is paid to the active sites of those molecules for which x-ray diffraction studies have provided detailed

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atomic structures. These structures serve as models for synthetic substitutes for the natural oxygen carriers that may have eventual medical application. Such uses of the results of studies on proteins that transport oxygen are discussed and the connections of these studies to other important biochemical processes are also noted.

Much of our understanding of biomolecular oxygen transport stems from structural studies on oxygen-carrrying proteins and much of this research has been carried out at the Naval Research Laboratory (NRL). The structures of four such molecules have been determined during the past few years as part of the research program of the Laboratory for the Structure of Matter at NRL. However, significant structural studies have been made elsewhere as well and chemical studies (such as the synthesis of analogs) from other laboratories have also contributed greatly to this area. Thus, this report reflects the participation of NRL along with many others in an exciting frontier of biochemical research.

Oxygen and Metabolic Efficiency

Stages of metabolic degradation

The role of oxygen is metabolism can best be understood in the context of an overview of the major biochemical pathways for the extraction of chemical energy from foodstuffs. This energy is needed to fuel various essential life processes such as muscular contraction for mechanical work, transport of ions through membranes during nervous activity and the synthesis of macromolecules for the perpetuation of life itself. In the case of green plants, the required chemical energy is generated from light energy by the process of photosynthesis. In the case of animals, the chemical oxidation of foodstuffs — fats, carbohydrates and proteins — provides the energy. Energy is extracted from the chemical bonds of the food molecules as they are degraded or "burned". The metabolic degradation of food molecules is performed in a series of many enzymatically catalyzed reactions. The series of biochemical reactions for the oxidation of foodstuffs can be conveniently grouped into three stages as shown in Figure 1.

The major foodstuffs are usually encountered as polymers built up of fundamental molecular components. Thus, in the first stage of metabolic degradation, these large molecules must be broken down to their constituent elementary units. Proteins are digested into the twenty different kinds of amino acids. Starches and other polysaccharides are broken down to single sugars, such as glucose. Fats are degraded to fatty acids and glycerol. There is no net release of energy in this initial fragmentation stage.

The second stage of degradative metabolism is one of unification. The various small molecule fragments derived from the foodstuffs are

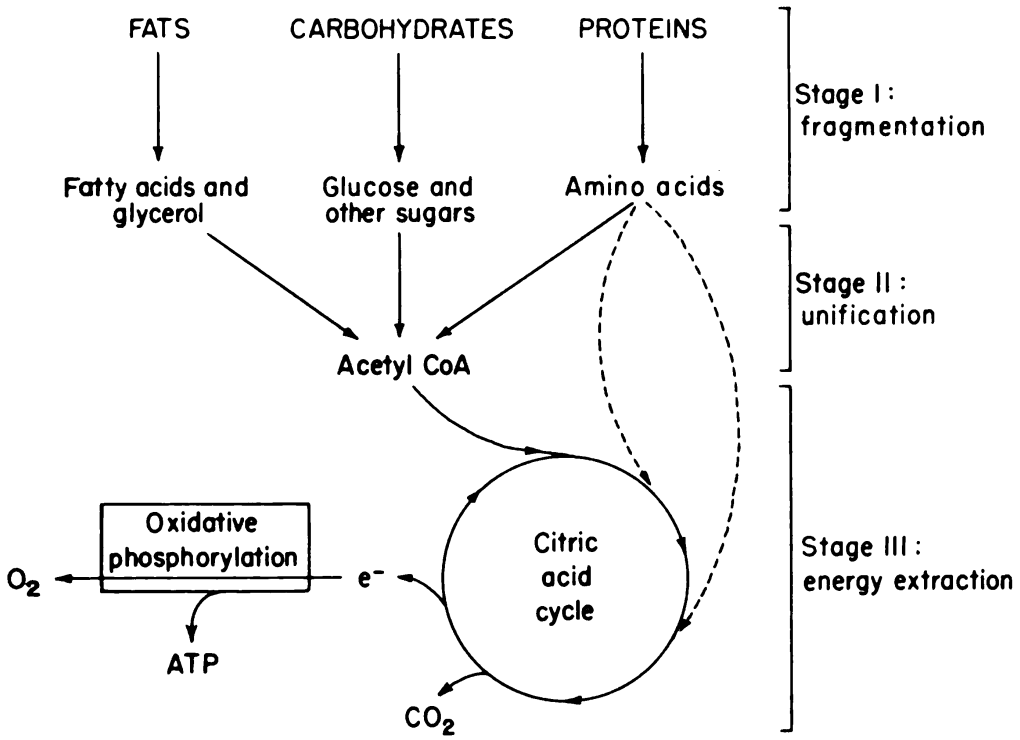

Figure 1 – Stages in the metabolic oxidation of foodstuffs. (Adapted from Stryer (1975) Biochemistry, Freeman.)

further degraded to simple components suitable for entry into a common energy extracting pathway. Sugars, fatty acids, glycerol and many of the amino acids are all converted, in a series of enzymatic steps, to acetyl groups linked to coenzyme A (CoA). Acetyl CoA has a high potential for enzyme-catalyzed transfer of acetyl groups. It is the universal carrier of acetyl groups and thus is a molecule of central importance in metabolism. Those amino acids that are not converted directly to acetyl CoA are degraded to other intermediates of the citric acid cycle.

There is some release of energy in the second stage of metabolism, but the final, oxygen-dependent stage is where the major energy extraction occurs. Acetyl units derived from the original food molecules are transferred from acetyl CoA into the citric acid cycle where they are completely degraded to carbon dioxide (CO_2). In the process, electrons are transferred to electron-carrying small molecules. These important electron carriers then inject electrons into the respiratory chain of reactions known as oxidative phosphorylation. The electrons are transferred through an assembly of intermediates to oxygen, which is the ultimate electron acceptor in this chain. As O_2 is reduced to H_2O, the electron carriers are reoxidized and this releases chemical energy that is packaged in the high-energy phosphate of adenosine triphosphate (ATP). The reactions of the citric acid cycle and oxidative

phosphorylation all occur within the membranes of specialized cellular organelles known as mitochondria.

Metabolic energy production

As has just been noted, energy extracted in the burning of foodstuffs is packaged as ATP (Adenosine TriPhosphate). So too is the energy derived from photosynthesis. The energy stored in ATP in turn, through the mediation of appropriate enzymes, drives all of the major life processes. ATP is used in the basic reactions of muscle contraction, in the active transport of ions and small molecules across membranes, and in the synthesis of both large and small biomolecules. Thus, ATP is a molecule of great importance to life on earth; it is the universal currency of metabolic energy exchange. The basis for this key role is the high potential for phosphate-group transfer by ATP. When enzymatically hydrolyzed, it releases inorganic phosphate and liberates a large, chemically useful quantum of energy.

So, the energy production from degradative metabolism can then best be reckoned in life's coin-of-the realm, ATP. Such an accounting makes clear the paramount importance of oxygen to animal life. The metabolic fate of a typical foodstuff, the starch from potatoes, exemplifies this point and is illustrated in Figure 2. Vegetable starch is a mixture of polysaccharides. These carbohydrates are broken down, without energy release, to glucose sugar units in the fragmentative stage of metabolism. Glucose is then degraded to pyruvate in a series of enzymatic reactions known as glycolysis. This generates two ATP molecules per glucose unit. In the absence of oxygen, pyruvate is converted to lactate (e.g. during muscle fatigue) or to ethanol (e.g. during vodka production), but no more energy is released. However, if oxygen is present and the pathways of the mitochondria are operative, acetyl CoA is formed from pyruvate and the citric acid cycle completes the degradation all the way to CO_2 while oxidative phosphorylation generates 34 additional ATP molecules per glucose molecule.

In sum, the complete biological oxidation of a polysaccharide of glucose (such as potato starch) yields 36 ATP molecules per glucose moiety. The total free energy stored in the 36 ATP molecules is 263 kilocarlories (kcal) (hydrolytic dephosporylation of ATP yields 7.3 kcal per molecule under standard conditions), whereas the chemical oxidation of glucose to carbon dioxide and water under standard conditions yields 686 kcal of free energy per molecule. This makes for an overall efficiency of 38% for the metabolic extraction of energy from potatoes. Since only 2 of the possible 36 ATP molecules are generated under anaerobic conditions, the energy harvest is enhanced 18-fold with the availability of molecular oxygen. Clearly, it is oxygen that is responsible for the high thermodynamic efficiency of metabolism. This accounts for the critical importance of oxygen to animal life.

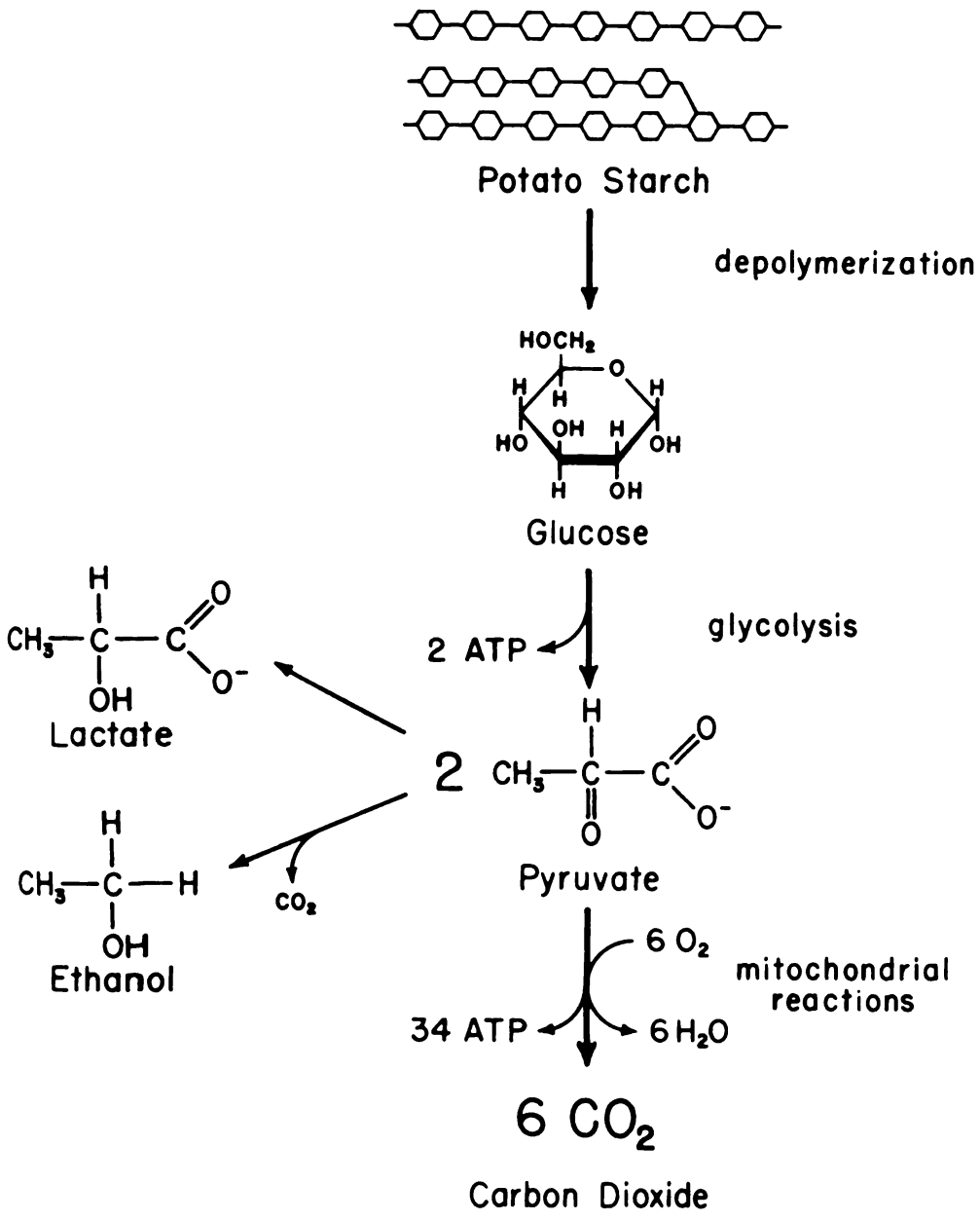

Figure 2 – Carbohydrate metabolism

Oxygen delivery

It is in the mitochondria that oxygen is used. Perhaps the most cogent demonstration of this is that cyanide quickly causes death. Cyanide specifically blocks the reactivity of cytochrome oxidase, the terminal element in the oxidative phosphorylation chain in mitochondria. Blockage of this enzyme directly prevents the reduction of O_2 to H_2O and brings the entire mitochondrial apparatus to a halt. Mitochondria are concentrated in tissues, such as those of muscle and brain, that require a ready supply of energy from ATP. Thus the body is faced with the crucial task of delivering oxygen, in plentiful supply, from the external environment to the tissues.

In small, simple organisms, such as bacteria or sponges, diffusion of oxygen through the cell suffices as a delivery mechanism. For most insects, an elaborate respiratory network consisting of air tubes has solved the delivery problem. However, the limitations of this system restrict the size that insects can ultimately achieve. The process of diffusion, whether through the cell sap or through airways, is too slow to meet the energy needs of large, active animals. Such animals have evolved circulating body fluids (blood) to actively transport oxygen from respiratory organs (gills or lungs) in contact with the external environment to the mitochondrial energy factories in tissues. Since oxygen is only sparingly soluble in water, special oxygen-carrying molecules are an essential ingredient of the blood of most animals. These molecules reversibly bind molecular oxygen and thereby effectively increase the solubility of oxygen in blood. The blood of man, for example, has its oxygen transporting capacity enhanced fifty-fold by the presence of hemoglobin, the oxygen carrier of humans.

The prime requirement for an oxygen carrier is reversibility of oxygenation. Oxygen bound in the lungs must be released unaltered in the tissues. This is a property that is not readily attained. The normal mode of reaction for oxygen in the midst of water is oxidation (for example, the rusting of iron). Oxygen withdraws electrons from the metal and O_2 irreversibly loses its identity. It is the need to counter this normal tendency for oxidative interactions that complicates the design of oxygen-carrying molecules. Hence, despite the simplicty of the O_2 ligand, these molecules are rather large and intricate metal-containing proteins. The protein chain is so folded as to sequester metal ions in a non-aqueous environment and form a metal complex that is electronically poised for reversible oxygen binding. These molecules are finely tuned marvels of bioinorganic chemistry.

Apart from reversible oxygenation, several other considerations affect the design of oxygen-carrying molecules. These molecules must be in plentiful supply to assure the necessary enhancement in oxygen capacity. Thus, they themselves have a high solubility in water. Moreover, they must not leak across the membranes of the circulatory

system nor cause, by their great numbers, an excessive osmotic pressure. Consequently, oxygen carriers either are packaged in high concentration within specialized cells or, if free-floating in solution, occur as giant molecules comprising many oxygen-binding sites. By contrast, the special oxygen-carrying proteins that facilitate the transfer of oxygen through muscles are rather small proteins adapted to the confining surroundings of muscle cells.

Oxygen-Carrying Proteins

Classes of oxygen carriers

In the course of biological evolution, nature has developed several alternative molecular devices to serve as oxygen carriers. Each of these is strikingly colored and this has perennially excited interest in their study. The visible absorption spectrum has also provided the main basis for classification of oxygen carriers. Thus, these proteins fall into three major families: hemoglobin, the familiar red substance in the blood of humans and many other animals; hemocyanin, the blue pigment in the blood of many molluscs and arthropods; and hemerythrin, the burgundy colored protein in the body fluids of a few minor invertebrates. In addition, certain annelid worms have green blood due to a hemoglobin-like protein that has a modified porphyrin group. The common prefix, *hem-*, in these names does not derive from the presence of heme (iron protoporphyrin IX) which occurs only in hemoglobin. Rather, heme and these proteins alike all take their names from the Greek work for blood.

There is considerable diversity in the structures of oxygen-carrying molecules. They vary greatly in size, symmetry and subunit composition. All are metalloproteins but each class is of a different sort. Hemoglobins contain iron by virtue of the heme group. Hemocyanins have dimeric copper centers and hemerythrins have dimeric iron centers. In these cases, the metals are coordinated directly by side chains of the protein. Even within a given family of oxygen carriers there are fundamental differences in molecular architecture. Structural studies make it quite clear that there is no homology between the amino-acid sequences or the three-dimensional foldings of the polypeptide chains of hemoglobin and hemerythrin. It also seems likely that none exists between hemocyanins and the others. Moreover, as is discussed below, there may well be more than one kind of hemoglobin fold and more than one kind of hemocyanin fold. Some of the properties of these three classes of proteins are summarized in Table 1.

Evolutionary distribution

The diverse natural occurrence of the different oxygen-carrying proteins shown in Figure 3 leaves few clues as to the course by which

Table 1. Some properties of oxygen-carrying proteins

	Hemoglobin	Hemocyanin	Hemerythrin
Active site			
Metal	$1Fe:O_2$	$2Cu:O_2$	$2Fe:O_2$
Coordination	Porphyrin and histidine	Protein side chains	Protein side chains
Color (oxy)	Red	Blue	Burgundy
(deoxy)	Red-purple	Colorless	Colorless
Distribution	a) Intracellular Widespread b) Extracellular i) Annelids ii) Molluscs iii) Crustaceans iv) Nematodes	a) Molluscs b) Arthropods	Sipunculans, brachiopods, priapulids & annelids
Structure			
Molecular weight	a) 6.4×10^4 b_i) 3×10^6 b_{ii}) 1.7×10^6 b_{iii}) 6.7×10^5 b_{iv}) 3.3×10^5	a) 9×10^6 b) 3.3×10^6	1.1×10^5
Size	a) 55 x 64 x 50Å b_i) 270 x 270 x 180Å b_{ii}) 120 x 120 x (?)Å b_{iii}) 130 x 130 x 85Å	a) 260 x 260 x 360Å b) 190 x 190 x 250Å	75 x 75 x 50Å
Symmetry*	a) C_2 b_i) $D_6 \times (?)$ b_{ii}) $C_6(?)$ b_{iii}) $C_5(?)$	a) D_5 b) $(?) \times D_3$	D_4

* The symbols C_2, D_2, D_3, etc. refer to the point group symmetry of an object, that is, the set of operations that can be applied about some central point to transpose the object into itself. Thus, molecules with cyclic point symmetry, C_n, have subunits arranged in head-to-tail fashion about an n-fold rotation axis, whereas molecules with dihedral point symmetry, D_n, combine an n-fold rotation axis with n two-fold axes evenly spaced at right angles to it.

these molecules have evolved. Certainly, the progenetorial complement of genetic information, the availability of metal ions, and the concentration of oxygen in the primordial atmosphere must have been influential factors. Whatever the evolutionary path, the products all seem to be physiologically nearly equivalent. Despite striking differences in molecular architecture and organism of origin, the various oxygen-carrying proteins fuction very similarly.

Hemoglobin is distributed widely, but often sporadically, throughout the animal kingdom. It, exclusively, is the oxygen carrier of vertebrates. It is found in the erythrocytes (red blood cells) and in the muscles (where it is known as myoglobin) of nearly all vertebrate species. It also occurs in animals from several invertebrate phyla including annelids, molluscs, arthropods, echiurans, echinoderms and

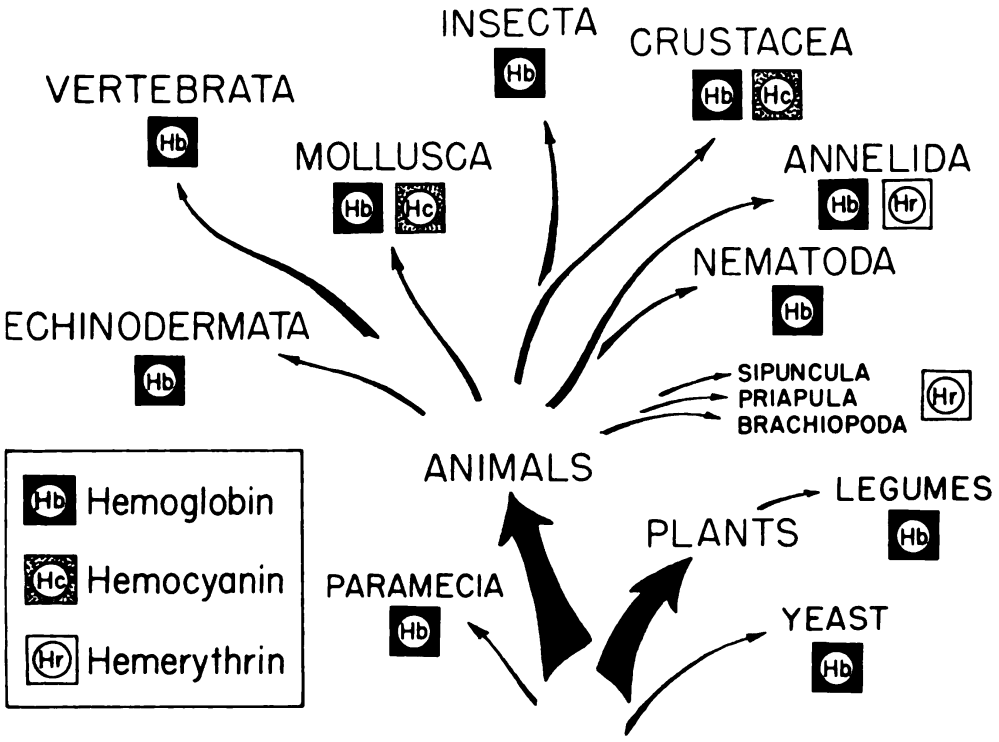

Figure 3 – Evolutionary distribution of oxygen-carrying proteins

nematodes, although sometimes it is represented in only a few of the genera. Among the invertebrates, hemoglobin occurs both in erythrocytes and as giant molecules freely dissolved in the blood. Some invertebrates also contain myoglobin. Hemoglobin is also found in the root nodules of legumes and in some molds, yeast and paramecia.

Hemocyanins are all giant molecules that occur free in solution. They are found in many molluscs (although not in clams) and in many of the arthropods other than insects. No myohemocyanin has been observed, but some hemocyanin-containing molluscs do have myoglobin. Despite functional and spectral similarities, the hemocyanins of arthropods and molluscs have distinctively different structural characteristics.

Hemerythrin is the least widespread of the oxygen carriers. It has been found only in animals from four invertebrate phyla: sipunculans, priapulids, brachiopods and annelids. Among annelids its occurrence is restricted to one minor genus. Three types of hemerythrins occur and all are intracellular proteins. One is carried in erythrocytes of the coelomic fluid, another is from red cells of the vascular system and a third, myohemerythrin, is found in muscle.

Intracellular hemoglobins

The hemoglobins that are found in erythrocytes and muscle have probably received the most intensive structural study of all proteins. Amino-acid sequences have been determined for dozens of globins, protein crystallography was nurtured to matuity on hemoglobin and myoglobin, and many other physical probes have been aimed at these molecules. As a result, the myoglobin fold and the subunit arangement in mammalian hemoglobin are so familiar that they scarcely need to be reviewed here. Suffice it to recall in Figure 4a the irregular disposition of helices surrounding the heme group of each subunit and the exact two-fold and pseudo-D_2 symmetry in the tetramer. On the basis of the uncounted structural studies on various mutants, chemically modified derivatives and ligand states of horse and human hemoglobin, detailed molecular mechanisms have been advanced to explain the cooperativity in action of this protein.

Hemoglobins from a diverse selection of other cellular sources have also been studied. Crystallographic structures have been determined for the myoglobins of the seal and tuna as well as for that of the sperm whale, and for the hemoglobins of the lamprey, an annelid worm, an insect and a leguminous plant. Despite as little as ten percent homology in sequence, the foldings of polypeptide chains in these structures are all remarkably similar. This would seem to establish the universality of the myoglobin fold in intracellular hemoglobins; however, such may not be the case. Yeast hemoglobin is a single-chain molecule of 50,000 daltons* that contains flavin as well as heme, and paramecium hemoglobin at a molecular weight of 13,000 is considerably smaller than other hemoglobins.

Extracellular hemoglobins

The extracellular hemoglobins (sometimes called erythrocruorins) that are found in certain invertebrates are built on a very different plan. These are giant molecules that comprise many functional units and often have molecular weights in the millions. They have not yet been studied by crystallography, but electron microscopy and biochemical analysis have provided some structural information. Four radically different kinds of macro-hemoglobins are known to exist. The most

*This dalton is a unit of mass equal to 1/16th of the mass of oxygen-16. It is used here to express the molecular weight of a substance.

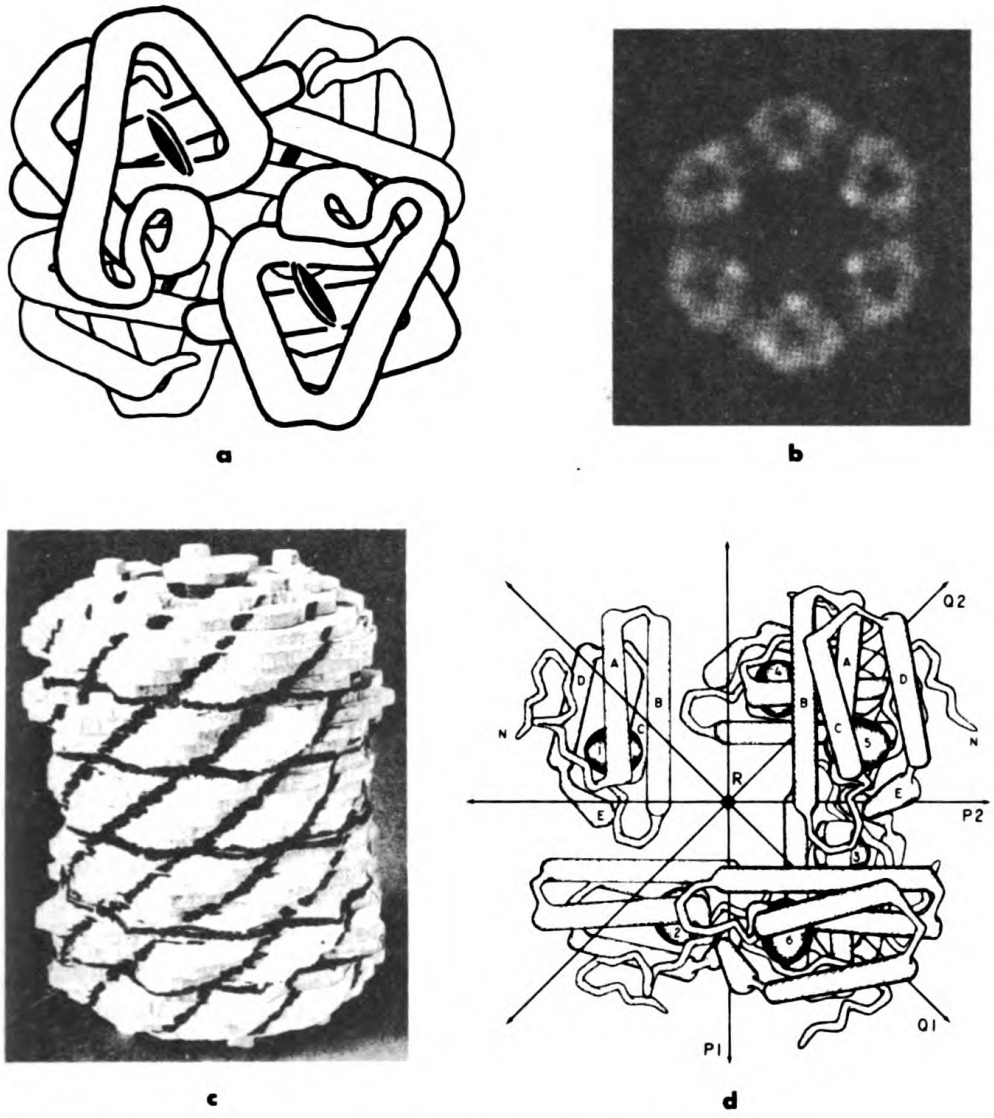

Figure 4 – Representative structures of oxygen carriers. (a) Human hemoglobin, (b) Earthworm hemoglobin, (c) Snail hemocyanin, (d) Peanut worm hemerythrin. (Reproduced from Hendrickson (1977) TIBS **2**, *108.)*

thoroughly examined kind come from annelids. These have a molecular weight of about three million and at least approximate D_6 symmetry. The molecules are arranged in two apposed layers of six-membered rings (Figure 4b) that can be dissociated into twelve major subunits. The exact composition of the 1/12th particle is still in question. However, the simplest proposal makes it a tetramer of equivalent units weighing 54,000 daltons each. These fundamental units are, in turn, complexes of two hemes and two copies each of two types of polypeptide chains that are linked together by interchain disulfide bonds. The helix content appears to be only about 40% as compared to nearly

80% for intracellular hemoglobins. Clearly these molecules are not simply high aggregates of human-like tetramers. Indeed, it is very possible that the heme is encapsulated by an entirely novel protein fold.

Much less is known about the structure of other extracellular hemoglobins. The largest molluscan hemoglobins also have molecular weights of a few million, but they are made up of very large polypeptide chains ($\sim$ 300,000 daltons) each of which binds twelve or more hemes. Each heme is associated with a mass of 17-22,000 daltons. Electron micrographs reveal ring-shaped molecules, probably six-fold symmetric, but of less than one-half the diameter of worm hemoglobins. By analogy with molluscan hemocyanin, it seems likely that these molecules may have a repeated domain structure. The third kind of large hemoglobins are those from branchiopod crustaceans such as brine shrimp and waterfleas. These are molecules with five-fold symmetry molecular weigths of up to half a million. The amount of protein per heme group is about 18,000 daltons, and the molecule can be dissociated into components of roughly that size. Finally, the hemoglobin found in the body fluid of nematodes is an octamer of 40,000-dalton units that each bear one heme group.

Molluscan hemocyanins

Molluscan hemocyanins typically have molecular weights of 7-9 million. These molecules can be dissociated into particles which are 1/2, 1/10 and 1/20 of the size of the complete molecule. The 1/20th particle is a single polypeptide chain with appreciably carbohydrate content and a molecular weight of some 300,000. This enormous chain can be cleaved by limited proteolytic digestion of native molecules into functional units of about 50,000 daltons, each of which contains one dimeric copper center for oxygen binding. In addition, the count of tryptic peptides gives evidence for an intrachain sequence repeat of at least 6-fold and electron micrographs of the 1/20th particles show a "string of beads" structure with 7-8 flexibly connected globules.

The data on subunit structure are compatible with three-dimensional reconstructions from electron micrographs of intact molluscan hemocyanin (Figure 4c). The whole molecule is seen to have D_5 symmetry and is constructed like a hollow cylindrical drum partially closed at the ends. The wall is of six quasi-equivalent layers each of which has approximate D_{10} symmetry. The collars that close off the ends are distinctly five fold. Thus the asymmetric unit comprises two doubled-up strings of beads that provide two half-length staves of the barrel wall and join intimately at the end to form one collar-piece. Each of the sixty quasi-equivalent wall units corresponds to a dimer of 50,000-dalton functional domains, and probably each collar-piece does also.

Arthropodal hemocyanins

The hemocyanins of arthropods provide yet another structural variation in oxygen carriers. These too are large molecules and have molecular weights as high as 3.5 million. However, the fundamental functional unit in this case is a single 70,000-dalton polypeptide chain which incorporates just one active center consisting of a coupled pair of copper atoms. All subunits seem to be quite similar, but extensive heterogeneity is typical. There is some evidence for internal duplication of sequence within the chains.

Under physiological conditions, these hemocyanins exist as polymers of the fundamental subunits. High pH and the removal of divalent cations tend to dissociate the native molecules. Analyses of the dissociation and reassociation behavior show that the first stage of association produces very stable hexamers. Higher aggregates form as dimers, tetramers or octamers of these hexamers. The ultimate state of association is species-dependent. Hemocyanin from the spiny lobster only forms hexamers. It has been crystallized and shown to have at least approximate D_3 symmetry. Hexamers of one kind of subunit isolated from one horseshoe crab have crystallographically exact D_3 symmetry. The shape and symmetry of the higher aggregates are not yet well understood, but these molecules certainly do not resemble their molluscan counterparts.

Hemerythrins

All hemerythrins have been found to be composed of subunits with molecular weights of about 14,000. Each of these is similar, if not identical, and is a functional oxygen-binding unit containing two iron atoms that are coordinated by protein side groups. Coelomic hemerythrins are typically octameric, but in certain animals they occur only as trimers. Myohemerythrin, however, is a monomer — a situation reminiscent of the relationship of myoglobin to hemoglobin.

The three-dimensional folding within hemerythrin subunits and the mode of subunit association in the octamers are now known from crystallographic studies (Figure 4d). Although hemerythrin, like hemoglobin, is a highly helical protein there is no further similarity in structure. The most salient features of the hemerythrin fold are four interconnected, quasi-parallel helix segments and the active iron center which they embrace. Local two-fold symmetry within the subunits further simplifies the structure. The octamer has crystallographically exact D_4 symmetry. Details of the molecular mechanism for reversible oxygenation in this system are just beginning to emerge.

Active-Site Structure

Hemoglobin

The structure of the oxygen-binding site in the intracellular hemoglobins is well understood. The prime component in this active center is the heme group, a non-protein prosthetic complement to the globin chain. Heme is the complex of iron with a prophyrin ring system. The porphyrin ring consists of four substituted pyrrole rings that are interlinked by methylene bridges. the arrangement is such that the nitrogen atoms of the pyrrole groups are perfectly placed to coordinate to an iron atom.

The heme group lies in a cleft, or pocket, between two helices of the folded globin chain. These helices are known as E and F. A covalent link from a histidine side chain on helix F fills the fifth coordination position of the iron atom. In oxyhemoglobin, O_2 occupies the sixth coordination position. Thus the iron atom is octahedrally coordinated as it commonly is in inorganic complexes. Many hemoglobins have a second histidine, this from the E-helix, near the active site. This distal histidine was once thought to be essential to oxygen transport activity, but the structures of the hemoglobins from an insect and from an annelid worm have proved this wrong. Other amino acids occupy the distal position in these proteins.

Although a single specific histidine is the only unique demand for the binding of heme by the globin, the provision of a pocket of hydrophobic character is an essential requirement. Residues from many and varied parts of the polypeptide chain come into van der Waals contact with the heme group. The specific environment of the heme group in lamprey hemoglobin is shown in Figure 5. The specific contact residues vary from hemoglobin to hemoglobin but the general nonpolar characteristics are always preserved. This is essential since heme in an aqueous environment reacts with oxygen by oxidation rather than reversible oxygenation. The nonpolar heme pocket protects the ferrous state of the iron by excluding water.

Hemerythrin

The active site structure in the non-heme oxygen carriers is less well characterized than is that in hemoglobin. In fact, the nature of the copper atom coordination in hemocyanins is essentially obscure at present. However, recent progress in the x-ray structure analysis of myohemerythrin makes possible a tentative description of the oxygen-binding site in hemerythrins.

Studies of the Mössbauer spectra, magnetic susceptibility data, and electronic absorption spectra from hemerythrins show that the iron atoms are antiferromagnetically coupled as in oxo-bridged iron dimers.

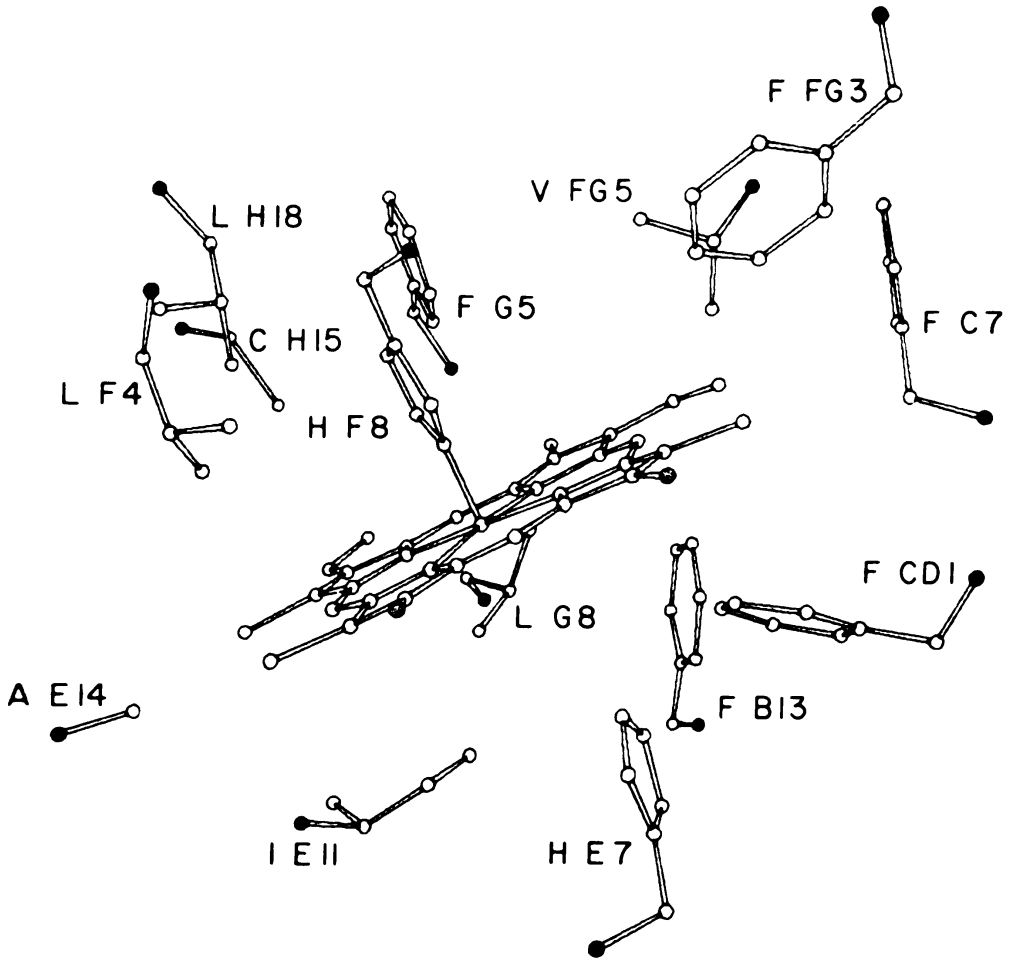

Figure 5 – Active-site structure in lamprey hemoglobin.
(Reproduced from Hendrickson et al. (1973) J. Mol. Biol. **74**, *331.)*

The x-ray determinations confirm that the iron atoms are close together at a distance, 3.4 A, that is compatible with model oxo-bridged structures. No prosthetic group is involved in the coordination of this dimeric iron center. Instead, side chains of the protein are bonded directly to the iron atoms.

The picture of the active site that emerges from the crystallographic study on the azide derivative of myohemerythrin is shown in Figure 6. A variety of side chain groups from diverse parts of the polypeptide chain are iron-linked. The functional groups that serve as iron ligands include the imidazole rings of five histidines, the phenolic oxygen of a tyrosine, and the carboxyl groups of a glutamate and an aspartate. The azide ligand and probably a water molecule complete the octahedral coordination about the iron atoms. It is reasonable to assume that oxyhemerythrin has an active site structure analogous to that shown in Figure 6, but the deoxyhemerythrin structure must be

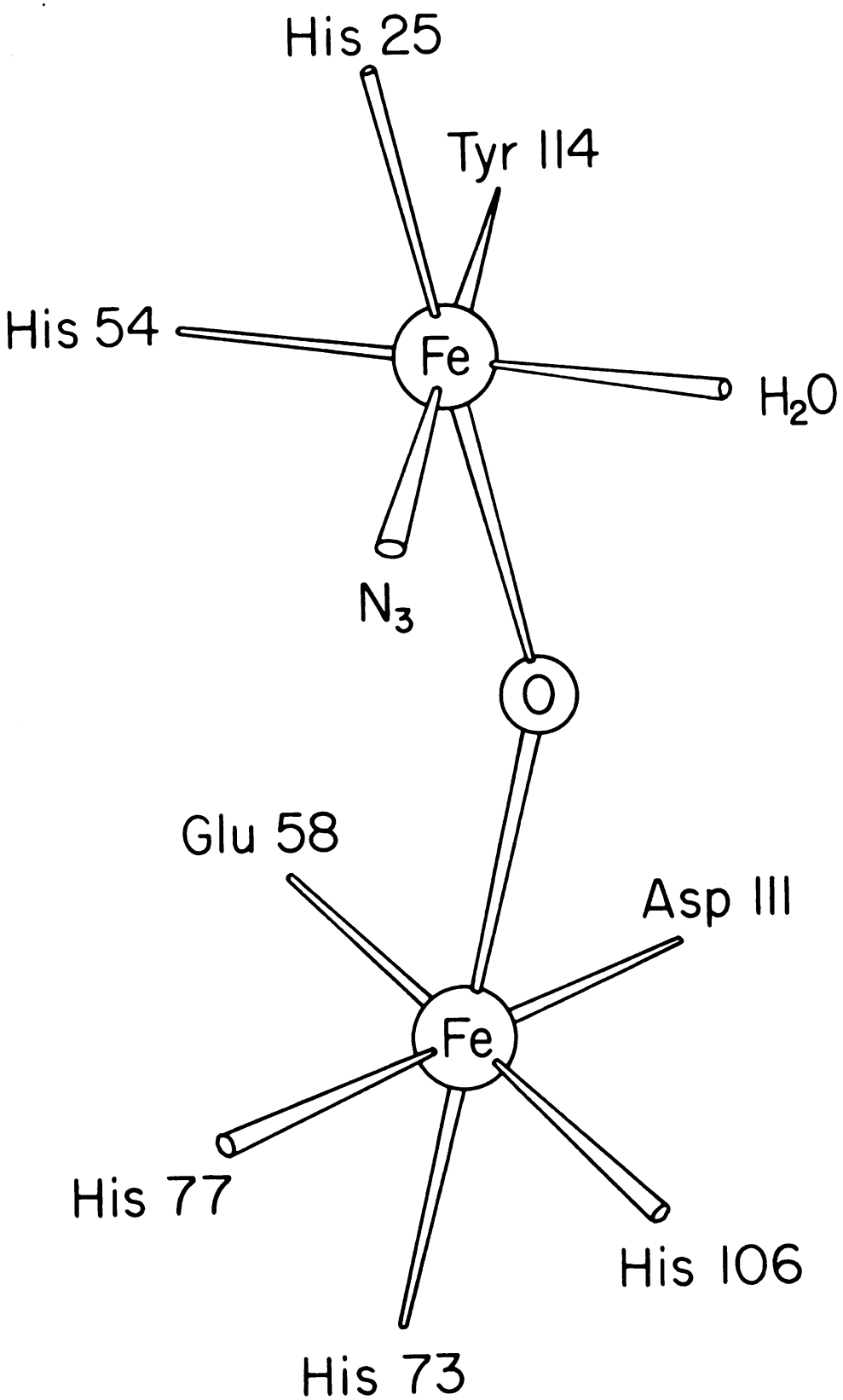

Figure 6 – Active-site structure in hemerythrin.

appreciably different. The iron atoms are not directly linked in the deoxygenated state.

The iron center of hemerythrin is insulated from the surrounding medium by a cage formed from the four major helices. Apart from polar iron-ligands, the active site in hemerythrin, like that in hemoglobin, has a hydrophobic character. The apparent oxygen-binding site is bounded by the nonpolar side chains of phenylalanine, tryptophan, leucine and isoleucine. A chink between the A and B helices affords an avenue of access to the binding site for extrinsic ligands such as oxygen or azide. This access-chink faces the large central cavity in octameric hemerythrin.

Applications and Connections

Synthetic analogs

Structural studies on hemoglobins have had practical impact in a number of ways. For example, knowledge of the three-dimensional structure of hemoglobin has been instrumental in the design of drugs for the treatment of sickle-cell anemia. It has also provided the conceptual framework for the clinical investigation of many other physiological disorders of the blood. However, perhaps the most striking role that the hemoglobin structural results have played is as a kind of blueprint for the laboratory synthesis of small molecules that can mimic hemoglobin activity. Such synthetic molecules can have very important medical applications in artificial respirators for use during surgery and as hemoglobin supplements in whole blood. Applications to the development of artificial gills for extracting the dissolved oxygen from water can also be envisioned.

The most successful of the several synthetic analogs of hemoglobin that have been devised is that known as Collman's picket-fence prophyrin (Figure 7). It has spectral properties like those of hemoglobin and it binds oxygen reversibly and repeatedly at room temperature. The synthesis of this molecule took cognizance of two essential features of hemoglobin structure: (1) the covalent linkage to the proximal histidine is the only specific interaction between heme and globin and (2) the oxygen-binding cavity is a protected hydrophobic cavity. The pickets of the picket-fence porphyrin are pivalamido groups substituted onto the ortho positions of tetraphenylporphyrin. The steric bulk of these pickets forms a hydrocarbon cavity for the oxygen ligand on one side of a heme-like plane while leaving the other side open for coordination to N-methylimidazole, an analog of histidine.

The developing picture of the active-site structure in hemerythrins promises to serve as model for an alternative class of synthetic oxygen carriers. Such molecules might have an important advantage for medical application. Hemoglobin has a binding site that reduces the high

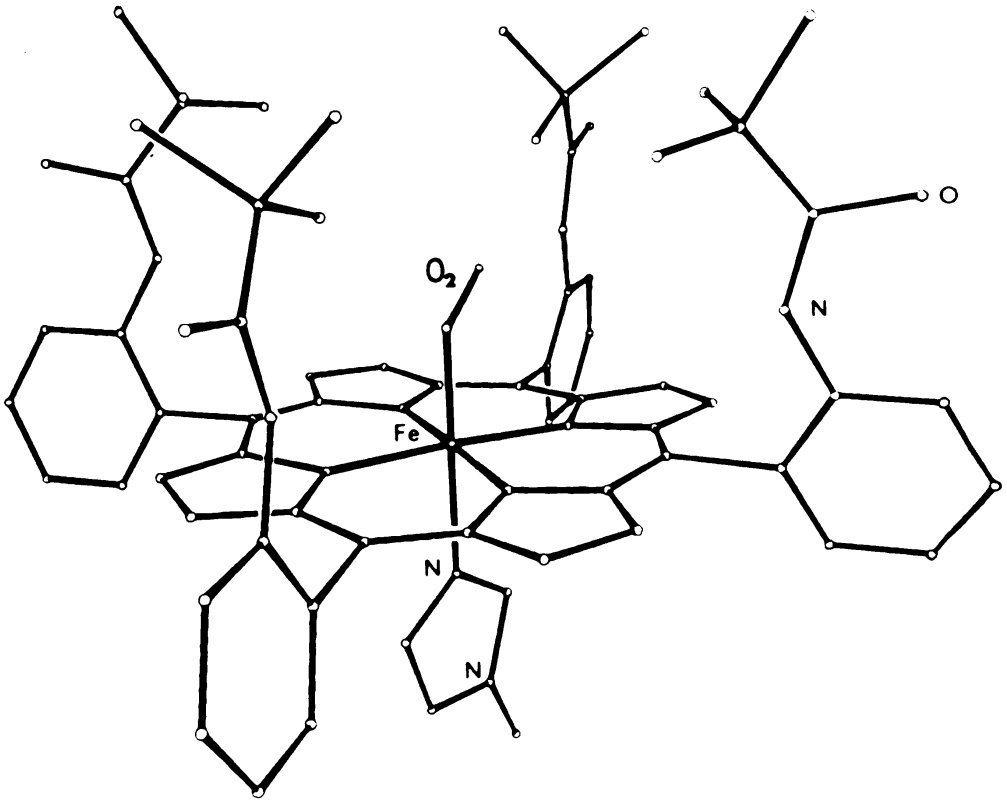

Figure 7 – Structure of the picket-fence prophyrin, a synthetic analog of hemoglobin. (Adapted from Collman et al. (1974) PNAS 71, 1326.)

intrinsic affinity of heme for carbon monoxide (CO), but the picket-fence porphyrin lacks this discrimination and would be poisoned by the combined endogenous and environmental presence of CO in blood. Since hemerythrin does not bind CO, its synthetic analogs would not be susceptible to this CO poisoning.

Cognate molecules

Nature tends to be parsimonious in the evolution of proteins. New needs are met by modifying or elaborating existing patterns rather than by building new ones from scratch. As a result, the structural themes present in the various oxygen-carrying proteins recur in what are seemingly otherwise unrelated molecules. Thus, the results of studies on oxygen-carrying proteins assume an importance that transcends the problem of oxygen transport. The structures of these molecules provide models, at a first-order approximation, for the nature of proteins with cognate qualities.

Many proteins besides hemoglobin make use of heme groups. These include cytochromes, catalase and peroxidase. However, the

mode of linkage to the protein often differs from that in hemoglobin. A pertinent example is cytochrome oxidase, the terminal enzyme in the respiratory chain, which has two modified heme groups. Interestingly, the iron atoms of the hemes become oxo-bridged as in hemerythrin on oxidation. Moreover, like hemocyanin, this enzyme complex also contains two copper atoms.

A closer analogy in structure appears to be present between hemocyanin and tyrosinase. Tyrosinase catalyzes the hydroxylation of phenols, such as tyrosine, to catechols which it then oxidizes to o-quinones, such as melanin. One effect of this ubiquitous enzyme is the aerobic browning of cut fruit. The close spectral similarity between these dimeric-copper proteins suggests a structural similarity at the active site. Indeed, this prospect prompted the discovery of a stable, reversibly oxygenatable form of the enzyme.

Perhaps the most significant parallel between an oxygen carrier and another protein is that between hemerythrin and ribonucleotide reductase. Ribonucleotide reductase is a kingpin in the replication of DNA, the hereditary molecule. It catalyzes the reduction of ribonucleoside diphosphates to the corresponding deoxyribonucleotides that are necessary precursors for DNA synthesis. This large, complex enzyme is controlled by an intricate system of feedback that regulates genetic replication. The enzyme has spectral and magnetic properties like those of hemerythrin by virtue of a dimeric-iron center that is essential to its catalytic activity. The hemerythrin model then affords some insight into the basis for this important activity.

Concluding Remarks

Advances in unravelling the diverse and intricate structures of molecules that carry oxygen have been great in recent years. Continuing progess is in prospect on the many challenging problems that remain. Such structural knowledge is essential to a solid understanding of the physiological roles that these molecules play. Moreover, these molecules are of interest as a study in evolution, as examples of very elaborate systems of self-assembly, as models of highly cooperative biochemical action, as subjects in the bioinorganic chemistry of reversible oxygenation, and as possible prototypes of synthetic substitutes for hemoglobin. It is also worthy of note that the molecular plans for these structure have probably been used elsewhere in nature.

Bibliography

Readers who wish to learn more about the subject molecules of this article are referred to a brief review by W.A.H. entitled "Molecular Architecture of Oxygen-Carrying Proteins" *(Trends in Biochemical Sciences*, V.2, pp. 108-111, 1977) and to the primary references listed

therein. Those desiring more information about the fundamental biochemical processes mentioned here are advised to consult the excellent new text by Lubert Stryer, *Biochemistry* (Freeman, San Francisco, 1975).

Stochastic Theory of Molecular Collisions

One of the most difficult problems in atomic and molecular physics, the deposition of energy in molecular collisions, is critical to the development of high efficiency lasers. The problems arise because for even low energy collisions of the order of several eV's there are a very large number of accessible states, there are many degrees of freedom (translational, rotational, vibrational, and electronic) to which energy can be transferred, and a number of mechanisms exist for energy transfer within and between the various degrees of freedom. In principle, the problem can be solved via a close coupling procedure involving a set of coupled differential equations. Since the computational difficulty goes approximately as n^3 where n is the number of states required, this method quickly becomes economically unfeasible.

Dr. H. Rabitz of Princton has formulated the problem statistically. This is accomplished by converting the expression for the wavefunction of the system at some time t in terms of the time evolution operator to an expression for the complex amplitude. From this one can obtain an exact expression for the probability of finding the system in a particular state at some time in terms of the complex amplitudes. Under very weak and very strong coupling conditions the cross terms in this expression, which can be oscillatory making computations difficult, can be approximately neglected. From this one can set up a difference equation wih respect to time to determine the probabilities for exciting various states during a collision. This research has been supported by the Office of Naval Research.

If one or more degrees of freedom have a large number of "closely" spaced quantum states available the master equation derived above can be converted to a partial differential equation (Fokker-Planck equation) in which the sum over these closely spaced states is replaced by partial differentiation. In effect, the energy of these states is treated as if it were a continuous variable. In fact, a system which contains degrees of freedom which cannot be treated as continuous states, as well as degrees of freedom whose states can be treated as continuous, can be represented by a mixed time dependent partial differential master equation, where the sum over the discrete states is retained. The complexity of the Fokker-Planck equation goes as the number of degrees of freedom and not as the number of quantum states available, which can result in a reduction of computer time of orders of magnitude.

Computer Security and Privacy

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Introduction

Computer security deals with the managerial procedures and technological safeguards applied to computer hardware, software, and data to assure against accidental or deliberate unauthorized access to and dissemination of computer system data. Computer privacy, on the other hand, is concerned with the moral and legal requirements to protect data from unauthorized access and dissemination. The issues involved in computer privacy are therefore political decisions regarding who may have access to what and who may disseminate what, whereas the issues involved in computer security are procedures and safeguards for enforcing the privacy decisions. The motivations for security and privacy can be found in the desire for secrecy in military affairs, for nondisclosure in industrial applications, and for information-sharing in modern society. These motivations have become particularly acute where computers are used since computers play a major and important role in processing and storing of secret and proprietary information and in providing effective sharing of useful information.

The relationships between privacy issues and security measures are depicted in Figure 1. By referring to Figure 1, we note that, through legislative measures, privacy issues affect all aspects of computer security. With due consideration of its social implications, legislation for computer privacy determines the type of information collected and by whom, the type of access and dissemination, the subject rights, the penalties, and the licensing matters. On the basis of the legislation, it

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*This is an abstract of the report "Research in Computer Security – Past, Present, and Future", by D.K. Hsiao, D.S. Kerr, and S.E. Madnick with an annotated bibliography by P. Sherburne. This ONR sponsored report in paperback assesses the current state of the art in computer security research in general and ONR contractors' contributions to this field in particular. After February 1978 this report is available from NTIS, U.S. Department of Commerce, P.O. Box 1551, Springfield, Virginia 22161.

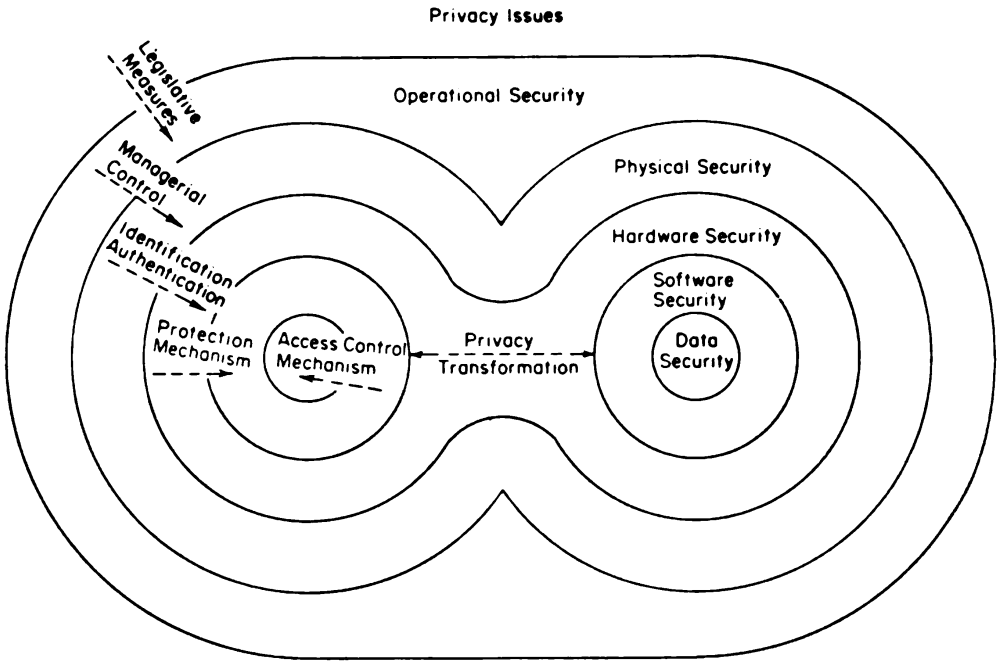

Figure 1 – A Computer System Environment consisting of two information Nodes.

is then possible to establish some form of operational security. The operational security allows the management of a computer installation to exercise control and be responsible for the installation. Guidelines and procedures may be established for accountability, levels of control, type of control (in terms of data classification and system configuration, information flow, and inventory), rules and checklists. Preventive measures and recovery due to internal threats and external intrusions are also a part of the operational security. For these threats and intrusions, the causes, effects, and means must be studied. More difficult aspects of operational security research include risk analysis, assessment, and insurance. By knowing the risks involved, the operational security may be expressed in terms of quantitative indicators, cost factors, and options. Finally, the dependability of the operational staff is necessary to successful operational security.

The Computers Physical Security

Through managerial control, the operational security allows the user to be physically close to the computer installation as depicted in Figure 1. Unless the computer system is physically secure, any further attempts to protect the computer system and system data will be futile. There are several areas of concern for physical security: control of physical access, prevention and recovery from loss due to natural disasters, electromagnetic and electronic tampering, and malicious entry and destruction.

With proper identification and authentication, a user may gain access to a computer system (again see Figure 1). Identification and authentication can be accomplished (1) via something that the computer knows (simple passwords, complex passwords, one-time passwords, handshaking through a question-answer session or dynamic program invocation, (2) via something that the user carries (keys, magnetic-stripe cards, or badges), or (3) via physical characteristics of the user (voiceprints, fingerprints or hand and facial geometry). Furthermore, actions upon intruder identification should also be considered as a part of the physical security.

Three Levels of Security

Once the computer system begins its work for a user, security is facilitated at three levels: the hardware level, the software level, and data level. In addition, if the computer system consists of terminals or several computers, then intercommunications between terminals and computers and among computers require security considerations.

Computer hardware security can be accomplished by means of (real and virtual) memory protection, multiple execution states, microprocessor, and minicomputers. In memory protection, access to areas of memory may be controlled by bounds registers (e.g., CDC 6000 computers), locks and keys (e.g., IBM 360 series), and access bits in real memory (e.g., tag-oriented architecture), in page table entries (e.g., UNIVAC system 70/46) and in segment table entries (e.g., IBM 370 series). The use of multiple execution states enables programs to be run not only in either supervisor or user state (where the supervisor state is endowed with more access privileges), but also in a hierarchy of states (where programs in a higher state are endowed with more access privileges than those in a lower one. Honeywell's Multics is a system with such hierarchies, known as protection rings). As hardware security aids, microprocessors may be placed between I/O channels and main memory for access control. They can be used as specialized processors for post-processing of data in order to enforce field-level and bit-level access control. As hardware security controllers, minicomputers may be used to perform periods processing — a DOD requirement for secure processing. They can also be used for monitoring the activity of a host computer. The monitoring may include the logging of the transactions and the alarming of the operator.

One of the main goals of the computer software security has been the design and implementation of secure software systems. First, we need a design methodology for secure software (e.g., the security kernel approach). Second, we must be able to verify and to test that the software produced is indeed the software intended (e.g., proof of correctness and penetration tests). Finally, we must have secure software which can carry out a wide range of security policies (say,

separation of policy and mechanism). Once we have learned how to design and produce secure software, we can then implement various software mechanisms to achieve desired security. There are essentially two types of mechanism available — those which rely on surveillance (such as logging, access control, and threat monitoring) and those which rely on isolation (such as virtual machines).

The most unique aspect of the data security is where some portion of the semantics of the data must be made confidential. Thus, the main concern in data security is safeguarding the confidentiality of the data semantics. To protect the confidentiality of the data, two principal problems must be resolved by the computer systems. The first is to conceal the data in user-computer and computer-computer communications (using privacy transformations); the second is to determine who can perform what operations on which data (providing access control). Privacy transformations, therefore, are techniques for encoding the data to hide its meaning. On the other hand, access control to a data aggregate requires the system to identify the user, to determine the data aggregate, and to enforce the authorized operations. Unless a user is properly identified, the system will not be able to establish the authorized data operations for the user. In order to determine the data aggregate on which the user is allowed to operate, the system must comprehend the content of the data. Without such comprehension, the system will not be able to determine the exact data aggregate involved. Thus, the semantics of the data plays an important role in access control. An "intelligent" access control mechanism of a computer system is one which can determine the proper data aggregate for access considerations despite the complicated semantics of the data involved.

Since data are handled as messages in user-computer (and computer-computer) communications, the classical cryptographic techniques have been used for privacy transformations. Here, the data are enciphered at the time and place of the entry. The encipherment involves the data, a key, and an operation. By performing the operation on the data digits and the key digits, the data entry terminal produces enciphered messages which will then be sent to the central computer system. An enciphered message returned by the system is deciphered at the data exit terminal. The decipherment involves the enciphered message, the same key, and the same operation. By performing the operation on the enciphered digits and the key digits, the data exit terminal produces the original data. Because both of the operations performed and the keys used at the data entry and the data exit terminals are the same, it is possible to use a single terminal for both data entry and data exit. Furthermore, the same operation may be built in the hardware, rather than software, for more rapid and reliable performance. However, the key (say, on a card with magnetic stripes) must be guarded by the user and changed frequently. Research has been directed toward the development of data terminals with privacy

transformation capability. Privacy transformations utilizing classical ciphers such as transpositions and substitutions (either monographic or polyalphabetic) have been used. However, frequent changes of keys require a way to produce random patterns of keys and there has been some research on the use of pseudo-random number generators for producing keys. When data bases are large and messages are long, there is a need for longer keys. Research into utilizing multiple short key tapes to produce a long compound key has been conducted. Because certain information (such as numerical data) is critical in some data operations and because errors in this information cannot be detected by context, there is need for a privacy transformation whose enciphered messages are sensitive to any change of a single digit position. Such privacy transformations not only can provide high message confidentiality, but also can provide and acute alert for the detection of errors. When data are required at the central site, the data aggregate may have to be deciphered either partially or completely for subsequent data management. This means that the system must have access to keys. A fundamental problem is therefore the capability of the system to protect the keys.

Access Control

In handling data in a computer system, the user will first want the data to be represented in formatted form in order to refer to it in terms of logical aggregates such as fields, arrays, tables, records, subfiles, and files. These aggregates are logical units of information which may have little resemblance to their physical or virtual storage images. By allowing the user to associate access control requirments and security measures with the logical units, the access control mechanism can facilitate direct control and protection of the data regardless of the location of that data. Furthermore, the mechanism does not require the user to be familiar with the physical or virtual storage structure of the computer system. For these reasons, such mechanisms in computer systems are referred to as logical access control mechanisms. Logical access control mechanisms must therefore have the facility for the user to specify shareable and private data in terms of logical aggregates of the data base, to assign access rights and security requirements to these aggregates, to determine the collections of these aggregates and the types of access that other users may have, and to incorporate additional authentication and checking measures.

The capability for the user to specify shareable and confidential data is directly related to the level of authorization and enforcement of the computer system. For example, some systems facilitate an authorization hierarchy which enables different users to have different rights for granting other access to the common data. Such a facility is particularly useful in a military environment where multi-level authorization

is a necessity. The level of enforcement is primarily reflected in its granularity. In other words, access control to large data aggregates, such as files, as well as small aggregates, such as fields, should be facilitated without severe overhead.

The determination of appropriate data aggregates for access control purposes may require resolution on the part of the computer system. Resolution is needed when more than one (possibly conflicting) security requirement is applicable to the same data. To provide automatic resolution, the system must have a known resolution policy (e.g., least disclosures and need-to-know policies). With the known policy, the system can then resolve the subsequent accesses to authorized data by either modifying te user request (known as the query-modification technique) or a posteriori checking (i.e., giving access only to those data which have been resolved for all applicable security requirements.)

Because the data base resides on secondary storage, considerations of data security also include compartmentalization of the data base. Ideally, data that have the same security requirement are stored in the same storage area (say, the same disk drive). Access to data satisfying one security requirement does not involve access to data satisfying other security requirments. In this way, both physical security and access control can be accommodated jointly for the data base.

In addition to the pass-through problem, data security also involves problems of inference even when the data are used only for statistical purposes. Since the data base is full of semantics, inference on the basis of this semantics can breach the confidentiality of the data. Again, the knowledge of the computer system in terms of the data semantics plays an important role in statistical and inferential control of data access.

Conclusions

In conclusion, we would like to emphasize that computer security and privacy covers a broad range of problems and issues which make the implementation of the security measures difficult and complex. Without a thorough understanding of the problems and issues involved, attempts to provide solutions in isolated areas may render the computer security inadequate. In this brief paper, we have tried to survey the entire spectrum of computer security and privacy so that its complexity and comprehensiveness are put in some perspective.

Research Notes

Mathematical Programming and Minicomputers

Professor Darwin Klingman, University of Texas at Austin, has recently completed an evaluation of the utility of minicomputers for mathematical programming applications. The study was motivated by the realization that efficient mathematical programming software on minicomputer systems would greatly increase the use of Operations Research (OR) throughout industry and government by placing decision making capability in the hands of managers. In fact, since minicomputers are relatively inexpensive, it might be possible to dedicate one to the solution of a specific recurring decision problem and design an operating system around the human engineering aspects of the problem.

The study indicated that algorithms for solving minimum cost flow networks are particularly well suited for minicomputers. In related research, Professor Klingman has demonstrated that a surprisingly large variety of optimization problems can be formulated as networks. The implementation of general linear, nonlinear, and integer programming algorithms does not appear practical at this time since their data and storage requirements are so large.

Professor Klingman has suggested several areas for further research. One is the development of algorithms that simultaneously use an array of minicomputers—each computer performing a specialized function within the algorithm. Another is the interface of information and optimization systems. Since the capabilities of minicomputers are greatly restricted, the simultaneous consideration of both systems in the design of a decision support facility is crucial.

A copy of the report can be obtained by contacting Dr. N.D. Glassman, Code 434 Office of Naval Research. Arlington, Virginia, 22217.

Unique X-Band Satcom Terminal

A team of Naval Research Laboratory scientists has developed and tested a unique interim high data rate (IHDRT) X-band satellite communication (SATCOM) terminal system for the Navy to use in transferring data from ships to shore facilities.

The system has already been installed on an oceanographic research vessel, where it is operating efficiently and meeting Navy requirements.

The Navy asked for a communications system that would reduce the size, cost and complexity of such systems in the future. The IHDRT program has met this objective and will provide cost management advantages for future Navy terminals.

Like previous SATCOM terminals, the system is a modular one but is integrated on the subassembly level using the best qualified sources. This has drastically reduced costs.

The concept for the new system allows improved cost management by the government because less manpower is required to design, fabricate, and test individual components, as they are specified, manufactured, tested, inspected, and finally integrated at the system level. More Navy ships may be equipped with the new SATCOM terminal in the near future. The research project was supported by the Naval Electronic Systems Command.

NRL Chemists find Laser-Catalyst Interactions Significant

Experiments at the Naval Research Laboratory have revealed a significant effect of catalytic reactions when carbon dioxide lasers are used to excite formic acid decomposition on various metal surfaces.

The results of the experiments suggest that lasers may be used with catalysts in the near future to produce new industrial chemicals and to effect isotope separation.

Although the mechanisms responsible for the observed effect is not yet fully understood, their observations strongly indicate the possibility of combining the unique properties of both catalysts and lasers to drive catalytic reactions in selected synthetic routes.

This could be an economical method for producing valuable chemical materials, including isotopically enriched substances such as heavy water (deuterium), which is used in large quantities in certain kinds of nuclear reactors.

The scientific team explains that formic acid decomposition on various metal surfaces (platinum, nickel, and silver) is known to produce carbon dioxide plus hydrogen and carbon monoxide plus water.

However, laser irradiation (9.6 μm CO_2 laser) of formic vapor near a platinum wire catalyst heated to 250 C enhanced the carbon dioxide/carbon monoxide product ration by some 50%. The effect was not altered by addition of a small amount of hydrogen, oxygen or by helium dilution.

Subcritical Crack Growth in AISI 4340 Steel

Coordinated fracture mechanics and surface chemistry experiments sponsored by the Office of Naval Research were carried out at Lehigh University to develop further understanding of environment enhanced subcritical crack growth in high strength steels. The kinetics of crack growth were determined for an AISI 4340 steel (tempered at 204°C) in hydrogen and in water, and the

kinetics for the reactions of water with the same steel were also determined. A regime of rate limited (Stage II) crack growth was observed in each of the environments. Stage II crack growth was found to be thermally activated, with an apparent activation energy of 14.7 ± 2.9 kJ/mol for crack growth in hydrogen, and 33.5 ± 5.0 kJ/mol in water. Fractographic evidence indicated that the fracture path through the microstructure was the same for these environments, and suggested hydrogen to be the embrittling specie for environment enhanced crack growth in hydrogen and in water/water vapor. A slow step in the surface reaction of water vapor with steel was identified, and exhibited an activation energy of 36 ± 14 kJ/mol. This reaction step was identified using Auger Electron Spectroscopy to be that for the nucleation and growth of oxide. The hydrogen responsible for embrittlement was presumed to be produced during this reaction. On the basis of a comparison of the activation energies, in conjunction with other supporting data, this slow step in the water/metal surface reaction was unambiguously identified as the rate controlling process for crack growth in water/water vapor.

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Cover Caption

Projected electron-density distribution for a crystal structure of the oxygen carrier hemerythrin at 5.5 A resolution. *(See Page 14)*

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