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Inclusion Compounds

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Keyphrases □ Inclusion compounds—polymolecular, monomolecular, blue-iodine reaction products, and macromolecular, review □ Complexes—review of various types of inclusion compounds □ Polymolecular inclusion compounds—urea and thiourea, choleic acids, 4,4′-dinitrobiphenyl, hydroquinone, water, phenol, Dianin's reagent, cycloveratril, and tri-*o*-thymotide, review □ Monomolecular inclusion compounds—cyclodextrins, review □ Blue-iodine reaction inclusion compounds—review □ Macromolecular inclusion compounds—zeolites, dextrans, and silica gels, review □ Urea and thiourea inclusion compounds—review □ Choleic acid inclusion compounds—review □ Water inclusion compounds—review □ Cyclodextrin inclusion compounds—review

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An inclusion compound is a unique form of chemical complex in which one molecule is enclosed within another molecule or structure of molecules. This combination is characterized by the absence of ordinary chemical bonds; the essential criterion is simply that the enclosed molecule or "guest" be of a suitable size and shape to fit into a cavity within a solid structure formed by "host" molecules (1–13). The hollow space formed by the host may be in the form of a channel, cage, or layer.

The stereochemistry and, possibly, the polarity of both the host and the guest molecules determine whether inclusion can occur. The resulting close fit of the two components produces a combination of significant strength due to the total dispersion forces between the interacting components. This type of spatial complex formation does not occur by means of ionic, covalent, or coordinate covalent bonds but rather is dependent upon dispersion forces and, possibly, highly oriented dipoles for stability, contrasting markedly with the usual concept of chemical complexation (14).

Inclusion compounds were first observed by Mylius (15) in 1886 as unusual complexations occurring between hydroquinone and several volatile com-

pounds. He proposed that the two components were interacting without chemical bonding and suggested that one molecule was enclosing the other. These observations were confirmed many years later by X-ray analysis; it was determined that one molecule of gas or liquid formed an insoluble inclusion compound with three molecules of hydroquinone, the host component, which formed a cage-like structure around the guest molecule (16). The presence of the guest component in inclusion compounds of this type could not be detected by odor; however, when the inclusion compound was heated or dissolved in water, the guest molecule was released.

The general title for this class of complexes, "inclusion compounds," was first used by Schlenk (17). Other terms that have been used to describe these complexes are "occlusion compounds," "adducts," and "clathrates" (18). Barrer (19) divided inclusion compounds into three categories, based on the varying concept of the host crystal, as *(a)* those that are stable both in the presence and in the absence of the guest molecule; *(b)* those in which the amount of guest may be changed but which have a critical concentration of guest molecules, below which the host structure becomes metastable and recrystallizes; and *(c)* those in which the host framework continuously readjusts itself as the content of guest molecules fluctuates.

A more convenient and workable classification, utilized in this review, is based upon the organization of inclusion compounds by their structure and properties as follows:

1. Polymolecular inclusion compounds
   —Channel-like spaces
   —Cage-like spaces
2. Monomolecular inclusion compounds
3. Products of the blue-iodine reaction
4. Macromolecular inclusion compounds

This classification differs from that of Baron (20), in that the products of the blue-iodine reaction follow the monomolecular inclusion compounds instead of appearing as the last category. This modification allows them to follow the classification to which they are more closely related.

Polymolecular inclusion compounds consist of a host structure composed of several molecules oriented in a loosely arranged lattice. The individual molecules of the host lattice are joined by hydrogen bonds to form a channel- or cage-like structure enclosing the guest molecule. However, they are not arranged in a whole number ratio to guest molecules as would be expected by the coordination theory (21). The inclusion cavity of specific dimensions forms only in the presence of the guest molecule but not necessarily using the guest as a template. In the absence of a guest molecule, compounds in this category form denser crystal structures without cavities.

As will be pointed out subsequently, similar molecules belonging to the same chemical class will crystallize to form cavities of different sizes and shape, depending on their atomic structure. Polymolecular inclusion compounds that form channel-like spaces include urea, thiourea, and various choleic acids; the latter complexes are among the earliest documented inclusion compounds (22).

A special category of the channel-like inclusion compounds consists of those formed by diphenyl derivatives in which the host molecules are "stacked" on one another in such a way as to leave channels in which an appropriate sized guest can be accommodated (23, 24). The interesting aspect of this arrangement is that no localized bonding is thought to exist.

The term "clathrate," derived from the Latin "clathratus," meaning "enclosed by the bars of a grating," (18) has been used to describe the cage-like structure of the hydroquinone inclusion compounds. The clathrates make up the second group of polymolecular inclusion compounds. In addition to hydroquinone, this group consists of: water or gas hydrates; tetraethylammonium hydrates; phenols; Dianin's compound, a product of the condensation of phenol and mesityl oxide; cycloveratril; and a number of complexes in which the cage-like structure is inorganic and the guest is organic (10). Although *Chemical Abstracts* and several other reference sources use the term "clathrates" as a general descriptor for inclusion compounds, this term more appropriately describes only the cage-like polymolecular inclusion compounds.

Probably the most versatile of the polymolecular inclusion compounds is tri-*o*-thymotide, which can form either channel- or cage-like void spaces, depending on the size and shape of the guest molecule (25).

The second major classification of inclusion compounds includes the monomolecular inclusion compounds. Although the polymolecular inclusion compounds constitute the largest group of inclusion compounds, the monomolecular inclusion compounds are of increasing interest and utility, particularly with reference to biological and pharmaceutical systems. Monomolecular inclusion compounds interact generally on a 1:1 basis with the guest molecule, which is enclosed within a cavity in the host molecule. The cyclodextrins, bis-*N,N'*-alkylenebenzidine compounds, antibiotics, and certain proteins are included in this category. The conclusion that certain proteins may behave as monomolecular inclusion compounds appears to offer a viable explanation for understanding antigen–antibody interactions, enzymatic reactions, and other shape-dependent processes.

Related to both the polymolecular and the monomolecular inclusion compounds are the products of the blue-iodine reaction. Iodine interacts with starch, cyclodextrins, flavones, coumarin, benzophenone, benzamide, cellulose, and barbituric acids to give a blue addition compound. This phenomenon has been attributed to the polymerization of iodine within unique channels formed by these compounds (26, 27).

The terms macromolecular inclusion compounds and "molecular sieves" have been used to characterize the fourth group of complexes. These compounds have been investigated extensively and have wide use in industrial and laboratory processes (19, 28, 29). Of

this group, the numerous zeolites are the most commonly known; however, the modified dextrans, polyacrylamide and agarose gels, silica gels, and other substances are also included.

The basic structure of the zeolites is a crystalline framework of silicon–oxygen or aluminum–oxygen tetrahedra which form a three-dimensional array with many cavities and interconnecting channels. Depending on the size and shape of these void spaces, various guest molecules can enter and be enclosed within the network. Although some macromolecular inclusion compounds occur naturally, it is also possible to custom tailor them to accept a specific guest by using the guest as a template during crystallization of the host.

Graphite is also generally classified as a macromolecular inclusion compound. However, it is more accurately classified as an intercalation compound, in which the guest is entrapped between layers of the graphite crystal (30, 31).

Inclusion compounds have been studied by a variety of conventional analytical methods including microscopy, IR and UV spectrophotometry, differential thermal analysis, NMR, and X-ray analysis[1].

Although a wide range of inclusion compounds is discussed here, emphasis is placed on those areas most apropos to the pharmaceutical sciences. Other areas are mentioned to present a comprehensive overview of this class of complexes. Previous reviews provided more extensive treatment of these latter areas (1–13).

POLYMOLECULAR INCLUSION COMPOUNDS

Compounds Forming Channel-Like Void Spaces—These inclusion compounds are classified as channel or canal complexes. Urea, thiourea, deoxycholic acid, and several other substances form long, tube-shaped cavities in which the guest molecules are oriented end to end.

Urea and Thiourea—Urea inclusion compounds were first reported by Bengen (32) from experiments on the effect of the addition of urea to pasteurized milk. Bengen was able to separate quantitatively the butterfat; however, in an attempt to control the formation of an emulsion at the oil–water interface, he added a small amount of *n*-octanol. After a short period, crystals were observed at the interface. These crystals later proved to be a urea–*n*-octanol inclusion compound. Bengen and Schlenk (21) were then prompted to study inclusion compound formation by urea and many aliphatic straight-chain hydrocarbons, leading eventually to the development of the use of urea inclusion compound formation as a method for separating hydrocarbons (33, 34).

In the presence of straight-chain hydrocarbons, urea generally forms a hexagonal structure surrounding a channel-like void space (Fig. 1) (17, 35–37), although the formation of a rhombohedral structure has been reported for some long chain hydrocarbons

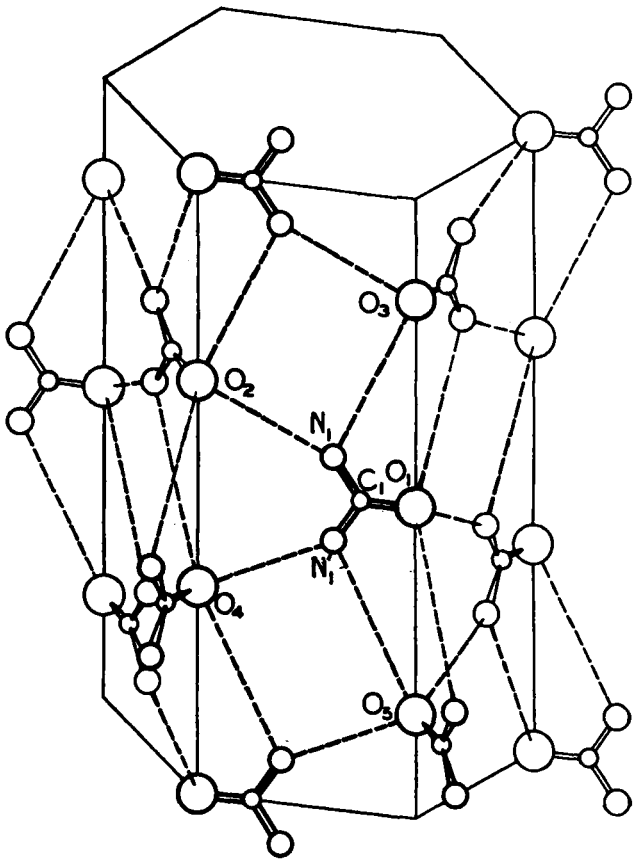

Figure 1—*Arrangement of hydrogen bonding in urea–n-hydrocarbon complexes. (Reprinted, with permission from Van Nostrand Reinhold Co., from M. Hagan, "Clathrate Inclusion Compounds," Litton Educational Publishing, 1962, and, with permission, from Ref. 36.)*

(38). The overall structure of the crystal is that of parallel tubes in which the hydrocarbon molecules are enclosed. The molecules of urea are held together in a loose network of hydrogen bonds between the nitrogen and oxygen atoms (36). The cavities have a diameter of approximately 5 Å, which is sufficient to accommodate straight-chain hydrocarbons and fatty acids or their derivatives. Nonwhole number molal ratios of urea to guest are found such as 6.3 moles of urea/mole of *n*-octane, 3.78 moles of urea/mole of methyl ethyl ketone, and 12.2 moles of urea/mole of oleic acid (39).

At room temperature, substances of a chain length of six or more carbon atoms form complexes with urea, while certain shorter chain-length compounds form urea inclusion compounds only at lower temperatures. In the absence of a guest component, urea crystallizes in a tetragonal arrangement. The structure is relatively open with low van der Waals interactions between the molecules (36). In the presence of a suitable size guest molecule, urea assumes the hexagonal arrangement around the guest, the combination producing a denser structure than that of the tetragonal arrangement. The inclusion compound is thus energetically favored. Schlenk (40) indicated an energy of formation of about 2.8 kcal/methylene group of the guest.

Several studies (34, 41) provided evidence for the

[1] These techniques were discussed in the review article by J. K. Haleblian, *J. Pharm. Sci.*, 64, 1269(1975).

solubilization of cetane, stearic acid, and *n*-valeric acid in saturated solutions of urea; this solubilization was not found for molecules that did not form urea inclusion compounds. The isomer of *n*-valeric acid, α-methylbutyric acid, does not form an inclusion compound and is soluble to the same extent in saturated urea solution as in water. The limited solubility of the isomer also indicates that the carboxyl group does not interact with the urea.

Many other substances form inclusion compounds with urea, including amino acids (42), epoxyesters (42), trioxane (43), aliphatic dinitriles (44), acrylic acid (45), and 1,2-bromotricosane (46). Urea inclusion compounds also have been used to separate racemic mixtures into optical antipodes (42, 47); the guest molecule may be collected by thermal or solvent decomposition of the inclusion compound (10, 42, 48). Fetterly (41) suggested that fatty acids dimerize in a urea inclusion compound, increasing the effective bonding by replacing a van der Waals bond by two hydrogen bonds. Thus, fatty acid–urea inclusion compounds are more stable in water solutions than the corresponding *n*-aliphatic hydrocarbon–urea complex.

As indicated earlier, the urea channel has a diameter of approximately 5 Å, limiting entrance to unbranched hydrocarbons. However, the sulfur analog of urea, thiourea, which also forms channel-like complexes, has a channel diameter of approximately 7 Å due to the larger size of the sulfur atom (36). A broad range of compounds can enter the larger thiourea cavity, including cyclic paraffins and polymethylated and polychlorinated hydrocarbons (49–51). Straight-chain hydrocarbons cannot form inclusion compounds with thiourea because the host framework is not stable and collapses. Since thiourea inclusion compounds do not give continuous layer lines in an X-ray pattern, it has been concluded that the guest molecule is fixed preferentially at certain planes in the channel.

The ability of urea and thiourea to differentiate between unbranched and branched molecules has led to significant industrial applications of these inclusion compounds (52–56). Numerous patents have been issued on processes utilizing the ability of the urea and thiourea inclusion compounds to separate mixtures effectively by removing molecules of a particular size and shape. Most of these applications are in the petroleum industry and involve the separation of hydrocarbon mixtures.

An example of such a process is the removal of high melting-point paraffins, which tend to solidify at low temperatures, from various oils. The straight-chain hydrocarbons are removed from the petroleum fraction by mixing the oil with urea and allowing the formation of the urea channel compounds. The inclusion compound is filtered or allowed to settle, and the supernate is decanted. Similar improvements can be made in other petroleum fractions where it is desired to remove naphthenes, terpenes, or other cyclic hydrocarbons. Both batch and fluidized-bed methods have been developed for these processes (34). The rate of inclusion compound formation is subject to the presence of inhibitors or activators (49, 57, 58).

The free fatty acids in acid-cracked lanolin can be removed as urea complexes, leaving neutral lanolin (59). The fatty acids can be collected by thermally decomposing the inclusion compound and then utilized for other purposes. The selective affinities of urea and thiourea can be used to improve ozokerites and other hydrocarbons of cosmetic and pharmaceutical interest (60).

One interesting aspect of urea and thiourea inclusion compounds is their use to increase the stability of easily oxidized substances. Vitamin A (61), unsaturated fatty acids (62), *d-trans*-oxocamphor (63), turmerone (63), decalin (64), and terpenes of orange oil and lemon oil and related compounds (65) have been stored as channel complexes. As long as the guest remains within the channel, it is protected from attack by atmospheric oxygen or from hydrolysis.

A novel solution to the handling of viscous, adhesive materials and potent or dangerous compounds is the formation of a urea or thiourea inclusion compound with appropriate substances in these categories. Liquid nonionic polyoxyethylene surfactants have been complexed with urea to form a solid powder which is easily handled (66–68). Straight-chain alkyl ethers, esters, and fatty acid derivatives of the polyoxyethylene surfactants form inclusion compounds, whereas sorbitan alkylate and alkyl aryl ethers of the polyoxyethylene series do not due to the large size of the sorbitan moiety and of the benzene ring. Likewise, the handling of potent insecticides such as chlordane is facilitated if the chlordane is in the form of a solid thiourea–chlordane inclusion complex (69, 70).

Cadwallader and Islam (71) prepared tablets of a dry, solid urea–benzalkonium chloride inclusion compound by direct compression.

Polymerization within inclusion compounds was first reported by Clasen (72) in 1956, who carried out stereospecific polymerization reactions of guest molecules within urea and thiourea host crystals. Brown and White (73) and White (74) irradiated monomers such as vinyl chloride and butadiene within their urea hosts and 2,3-dimethylbutadiene, 2,3-dichlorobutadiene, and 1,3-cyclohexadiene in a thiourea complex to form their respective polymers. The channels acted as smooth, straight templates holding the guest monomers, producing 1,4-*trans* addition polymers. The polymers obtained were crystalline and had higher melting points than polymers obtained by irradiation of the liquid monomers. Ivanov *et al.* (75) polymerized piperylene in the channels of urea and reported that the channel polymer had a higher degree of unsaturation (95–98%) than did polymers formed by other means (88%).

The formation of radicals during γ-irradiation of hydrocarbons (76), alkyl halides (77), and fatty acids (78) was recently reported.

The major limitation to this polymerization process is the limited number of molecules of proper size and shape able to reside in the urea or thiourea channels. The use of the channel as the template for the polymerization requires a reasonably precise fit of

the monomer within the channel.

Choleic Acids—The second group of polymolecular inclusion compounds forming channel-like void spaces are the choleic acids. Deoxycholic acid, a bile acid, forms channel-like inclusion compounds with numerous compounds including carboxylic acids, esters, alcohols, ethers, phenols, and hydrocarbons (79, 80). Apocholic acid (8,14-dehydrodeoxycholic acid) also forms similar inclusion compounds; however, the other naturally occurring bile acids are not known to form these complexes, apparently due to the absence of hydroxy groups at C-3 and C-12 (81).

Wieland and Sorge (22) first reported the composition of a choleic acid. Two products were evolved by thermal decomposition of the choleic acid *in vacuo:* deoxycholic acid (92–94%) and a waxy mixture of stearic and palmitic acids (6–8%). Excess palmitic, stearic, and oleic acids can be added to hot alcoholic solutions of deoxycholic acid to precipitate choleic acids of the same solubility and melting point, which can be distinguished only by crystallographic analysis (82). Fourier analysis of the structure of the deoxycholic acid–palmitic acid inclusion compound indicated that the deoxycholic acid molecules formed a framework around the axis of the guest molecules in which the host molecules were linked by hydrogen bonds (82); the host and guest molecules interacted *via* van der Waals forces. Similar results were found by Fourier analyses of choleic acids formed with other fatty acids. Since the choleic acids of C_4–C_{26} normal fatty acids had identical unit cells, they could not be coordination compounds.

Cramer (83) later indicated that a choleic acid is an inclusion compound with fatty acid guest molecules oriented in an end-to-end arrangement within the open channels of the deoxycholic acid crystal. An interesting aspect of the combination of these two unreactive components is that the nature of the molar ratio of the components differs from that in the urea–hydrocarbon inclusion compounds. Nonwhole number ratios were determined for the latter complexes, whereas the choleic acids are characterized by whole number ratios such as four molecules of deoxycholic acid to one of C_4–C_8 acid, six to one of C_9–C_{14} acid, and eight to one of C_{15}–C_{29} acid.

The preparation of choleic acids is not a difficult process. Both host and guest are dissolved in an appropriate hot solvent, *e.g.,* absolute methanol or ethanol, and then slowly cooled to permit crystallization of the choleic acid. The crystals can then be collected and washed, using small amounts of solvent to avoid dissociating the complex. It must be kept in mind that the choleic acids are readily dissociated in water.

The properties of certain substances are modified when they form choleic acids (81). Not only is the resistance to degradation increased, but other properties are influenced as well. For example, ethyl acetoacetate in liquid form or in solution contains 8–13% of the enol form, whereas it is 100% enolized in its choleic acid form. Likewise, acetylacetone is 70% enol in solution and 100% when complexed with deoxycholic acid. When the choleic acid crystals are dissolved, they dissociate and the liberated enol is ketonized at a measurable rate until tautomeric equilibrium is achieved. Racemic mixtures also have been separated by selective choleic acid formation; partial separations have also been accomplished for camphor, *sec*-butylpicramide, methylethylacetic acid, and α-terpineol (84, 85).

The stabilization of autoxidizable compounds by formation of a choleic acid has proved to be a fruitful area, producing inclusion compounds that are highly resistant to degradation not only by oxidation but also by heat and by chemical attack. Choleic acids of vitamins K_1 and K_3 were prepared (86), and complexes of vitamins D_2 and D_3 with deoxycholic acid and apocholic acid were patented (87). Crystalline choleic acids were formed that had vitamin activity equal to that of the uncomplexed vitamins but were significantly more stable with time. Likewise, stable choleic acids with high vitamin activity were prepared from vitamin A and vitamin A palmitate (66, 88–90).

One such crystalline choleic acid, "gallesterol," prepared by treating an egg-yolk preparation with deoxycholic acid, contained approximately 16.8% of highly stable vitamin A (91). The stoichiometry of the vitamin A choleic acids has been variously reported as one molecule of vitamin A to five (91) or eight (92) molecules of deoxycholic acid. By contrast, choleic acids of β-carotene, a precursor to vitamin A, have been reported (93) to be four molecules of deoxycholic acid per molecule of β-carotene. Other stable choleic acids include those prepared from deoxycholic acid and linoleic acid (88), linolenic acid (88), methyl linolenate (88), cinnamaldehyde (88), pentaerythritol tetranitrate (94), 2-methyl-1,4-naphthoquinone (95), and various aromatic solvents (96).

The earliest pharmaceutical use of choleic acid complexes was the preparation of stable choleic acid inclusion compounds of camphor with deoxycholic acid ("codechol") and apocholic acid ("camphochol") (97). The complexes were determined to have a guest–host ratio of either 1:1 or 1:2. "Cholosulin," the choleic acid of insulin and deoxycholic acid, was prepared and investigated (98); the absence of a pharmacological effect of the orally administered complex was reported. There is no direct evidence of the bioavailability of the choleic acids, although it has been indicated that choleic acids of streptomycin sulfate and of dihydrostreptomycin sulfate and its base could be formulated in solution, parenteral, emulsion, powder, tablet, and ointment dosage forms (99). One does not necessarily question the stability of the complex and of the guest molecule in the including structure; however, in the absence of evidence to the contrary, the bioavailability of the drug from such preparations must remain questionable and would be contingent upon the rate of dissociability of the complex and the site and conditions under which it occurred.

Indeed, such evidence was provided on the retardation of salicylic acid excretion after the oral administration to humans of a suspension of a deoxycholic acid–salicylic acid inclusion compound (100). The bioavailability of salicylic acid from the choleic acid

was less than that of salicylic acid from the administration of an equivalent dose of salicylic acid in solution. The difference in bioavailability was presumably due to the acid stability of the choleic acid. Micronization of the choleic acid increased the bioavailability of the salicylic acid. The stability of choleic acids also was studied under tablet manufacturing conditions (101).

Deoxycholic acid is limited in aqueous solubility, but its salts are highly water soluble. The salts are surface active, and micellar solubilization of lipids by these compounds is considered to be part of the mechanism by which fats are absorbed from the GI tract. It would be expected that a choleic acid would be reasonably stable at gastric pH but unstable as pH increased. The guest molecule would then be released in the intestine at a pH-dependent rate. Depending upon the site at which this release occurs, the guest may or may not be well absorbed, if it is absorbed at all. Since the bile salts, including deoxycholate salts, are believed to form cylindrical (channel-like) micelles containing solubilized lipids (102), in the broadest sense, micellar solubilization may be considered to be a form of polymolecular inclusion in solution, albeit that the nature of the interaction of the solubilizate, the guest component, with the surfactant host molecules does not necessarily fit the generalized "no bond" mechanism of the host–guest interaction characteristic of inclusion compounds.

Except for lamellar structures, micelles do reach a limiting size and shape, dependent upon the nature of the solubilizate, surfactant, and solvent. The surfactant monomers within the micelle are joined by van der Waals forces and by electrostatic bonds to form an essentially two-dimensional liquid crystalline structure enclosing (enclathrating?) the solubilizate. Thus, in the case of the choleic acids, one form of an inclusion compound may be exchanged for another, depending on pH and other conditions.

Several other steroids are thought to form inclusion compounds. Mesley (103) reported the inclusion of chloroform within lattices formed by hydrocortisone, dexamethasone acetate, and prednisone.

4,4′-Dinitrobiphenyl—These polymolecular inclusion compounds are formed by 4,4′-dinitrobiphenyl with certain 4,4′-substituted biphenyl derivatives (104). 4,4′-Dinitrobiphenyl molecules were described as being stacked in a face-centered arrangement so as to form channels that enclose the guest molecule (105, 106) (Fig. 2). The abundance of π-electrons electrostatically influences the guest molecules, which are generally nucleophilic and include benzidine, biphenyl, 4-bromobiphenyl, 4-iodobiphenyl, 4-hydroxybiphenyl, 4-aminobiphenyl, *N,N,N′,N′*-tetramethylbenzidine, 4,4′-dimethoxybiphenyl, 4-acetoxybiphenyl, and 4,4′-diacetoxybiphenyl.

Compounds Forming Cage-Like Void Spaces—Inclusion complexes formed by compounds in this category consist of a cage-like structure entrapping a guest molecule. The individual host molecules link together to form a cage or a cup-shaped structure, the open end of which joins with the open end of a second similar structure to form the cage. Because of the characteristic structure of these inclusion compounds, Powell (18) named them clathrates.

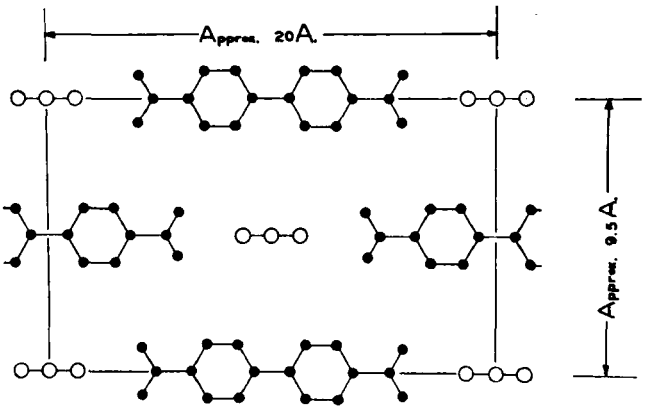

Figure 2—*Cross section of an idealized structure of the dinitrobiphenyl adducts. (Reprinted, with permission from the Eastman Kodak Co., from Ref. 24.)*

Hydroquinone—The hydroquinone–hydrogen sulfide complex was first described in 1849 (107). Mylius (15) later determined that whatever bonding was occurring in the hydroquinone–formic acid complexes he was investigating, it was not of the conventional types. He concluded that one molecule was enclosing another in a relatively stable structure. Examples of this class of inclusion compounds are those formed between hydroquinone and methanol, sulfur dioxide, hydrogen sulfide, hydrogen chloride, or other substances of a suitable size and shape.

Powell (18, 108–112) extensively studied the formation and properties of these complexes. He determined that six hydroquinone molecules are linked by hydrogen bonding to form a large open structure, which may be visualized as consisting of two interpenetrating "cups," each cup formed by three hydroquinone molecules. The hydroquinone molecules in each cup are hydrogen bonded at one end, with the molecules tilted at an angle outward from a plane perpendicular to the hexagonal plane of the hydrogen-bonded hydroxy groups (Fig. 3). The open ends of two such cups interpenetrate to form the cage. Alternate hydroquinone molecules incline upward or downward from a plane hexagon formed by the six oxygen atoms of the six hydroquinone molecules.

Hagan (10) and Brown (11) published excellent diagrams of these structures. The individual hydroquinone structures are not chemically bonded to each other but form an infinite framework. The cage-like framework encloses void spaces approximately 4 Å in diameter, from which the guest molecules cannot escape. Hydroquinone clathrates are formed only by molecules that can fit inside the cavity; molecules small enough to slip out of the cage do not form a clathrate.

Hydroquinone and similar clathrates are prepared by saturating a solution of the host component with the guest. The framework gradually develops and the clathrate forms as a crystalline solid. Some systems are more difficult to prepare and require high gas pressures and/or temperature adjustments to ensure

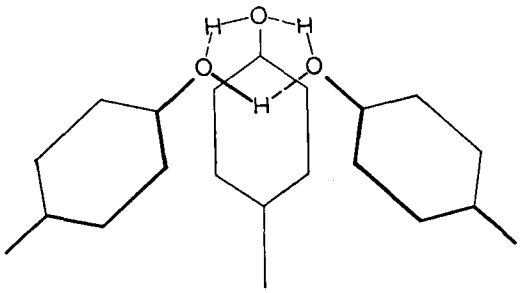

Figure 3—*"Cup" of three quinol molecules formed by hydrogen bonds. (Reprinted, with permission, from Ref. 6.)*

the desired ratio of host to guest to initiate crystallization. A less than stoichiometric ratio may result, indicating that not all of the void spaces are occupied. For a clathrate to form, the guest must be in place when the host framework forms. It is also possible that the solvent may be included if of a suitable molecular size. Solvent clathrates form in the absence of any other guest compound or, alternatively, the solvent molecule may compete with a suitable guest for inclusion.

The crystals that form are very stable; odors, if any, of guest gas molecules cannot be detected. However, the clathrate may be dissociated by heating, by dissolution in water, or by crushing or grinding the crystals (113–115).

Numerous compounds form clathrates with hydroquinone including gases, thiophene, benzene, furan, pyrroles, phenol, hydrochloric acid, and methane (6, 10, 12). The important criterion is the magnitude of the molecular volume of the guest component relative to the volume of the cage; that is, the clathration process is essentially physical in nature, not chemical.

Studies of the thermodynamics of the formation of hydroquinone clathrates of methanol and sulfur dioxide have indicated that the heat of formation of these clathrates is zero and that the free energy differences between the clathrates and the pure components is entropic in nature (116). Aside from crystal lattice interactions, the interactions of the guest and host molecule in hydroquinone clathrates are the only significant forces (117). Evans and Richards (118) suggested that such clathrates thermodynamically resemble dilute solutions.

Van der Waals and Platteeuw (119) proposed a statistical mechanical treatment for the clathrate system, consisting of three phases in equilibrium: clathrate, nonclathrate modification of host (polymorphic changes), and pure nonpolar gas, assuming only one guest molecule per host void space. The guest molecule can move within the cage, but the potential energy of the guest does not entirely depend on the sum of the two terms depending on the potential and orientation coordinates but also on the vibrational state of the molecule moving in its cage. The rotational contribution to the free energy depends on the vibrational state; the molecule loses orientational freedom when enclathrated, and this effect is greater for polyatomic molecules than for nonatomic gases.

Hydroquinone clathrates have found use in extractive crystallization by selective clathration. Rare gases are good examples. Argon may be separated from neon by adjustment of the conditions under which the clathrate forms to conditions specific for one gas or the other, and krypton may be separated from xenon (120). Selective clathration offers great potential for the removal of suitable solutes from solution and for the recovery of impurities or trace compounds.

The good storage capability and ease of handling of the hydroquinone clathrate crystals were utilized in a novel method for the handling of radioactive krypton, ^{85}Kr (121). The dry, crystalline hydroquinone–^{85}Kr clathrate was easy to handle when compared to the use of the heavy, bulky cylinders normally used to store this gas under pressure. The clathrate crystals produced radiation approximately 25 times as intense as that of an equal volume of gas at atmospheric pressure. The clathrate forms an almost leakproof container for the ^{85}Kr gas, which can be powdered and compacted with a minimal loss of radioactivity. These stable radioactive sources find a number of applications wherever ^{85}Kr is used to eliminate static and measure thickness and in other uses of β-radiation such as those of Schuler *et al.* (122) who measured acute changes in regional blood flow by intramuscular administration of ^{85}Kr clathrate.

Water—Under certain circumstances, water molecules form a crystalline clathrate framework containing cavities in which gases or low boiling-point liquids can be entrapped. Such clathrates are termed "gas hydrates" or "liquid hydrates." Compounds with chemically bound water of crystallization are also called hydrates but differ from clathrates in the nature of the bonds holding the water and in the structural arrangement of associated water molecules.

Davy first reported the chlorine hydrate in 1811. Subsequently, Faraday, Lowing, and others analyzed this complex and others, but much controversy raged concerning the exact nature of the inclusion complexes (123). It was not until X-ray diffraction studies of the gas hydrates that the water clathrates were determined to be solid solutions of a gas or a gas contained in a metastable water lattice.

The water clathrate framework resembles the ice lattice in that each oxygen atom is hydrogen bonded to four other oxygen atoms. The hydrogen bonds in ice have a length of 2.76 Å, virtually the same as in the clathrate (2.75 Å). X-ray diffraction studies have shown that water molecules in the clathrate framework are joined mostly in rings of five molecules rather than of six as in ice (124–134). The pentagons are oriented to form dodecahedrons, which are the "building blocks" of the clathrate structure. Since the dodecahedrons cannot be perfectly packed, void spaces are formed; it is in these spaces that the guest molecules are held (Fig. 4). Ordinary ice also has void spaces, but they are not of sufficient capacity to hold molecules larger than helium or hydrogen.

There are three classes of water clathrates, but the first two are most often referred to in the literature

Table I—Polyhedral Hydrates[a]

Class I	Class II	Class III
Guest Molecules		
Ar, Kr, Cl_2, H_2S, PH_3, SO_2, $C_2H_5NH_2$, $(CH_3)_2NH$, CH_2CH_2O, $CH_2CH_2CH_2O$, CH_4, C_2H_2, C_2H_4, C_2H_6, CH_3Cl, CH_3SH, CH_3CHF_2, CH_2CHF	$CHCl_3$, CH_3CHCl_2, $(CH_3)_2O$, C_3H_8, $(CH_3)_3CH$, C_3H_7Br, $(CH_3)_2CO$, C_6H_6, $CH_2CH_2CH_2CH_2O$, cyclo-C_6H_{12}, $CH_2CH_2OCH_2CH_2O$	$(n\text{-}C_4H_9)_4N^+F^-$, $(n\text{-}C_4H_9)_4N^+{}^-O_2CC_6H_5$, $[(n\text{-}C_4H_9)_4N^+]_2WO_4^{2-}$, $(\text{iso-}C_5H_{11})_4N^+F^-$, $(n\text{-}C_4H_9)_3S^+F^-$, $(n\text{-}C_4H_9)_4P^+Cl^-$, $(CH_3)_3N$, $(CH_2)_6N_4$, $n\text{-}C_3H_7NH_2$, $(CH_3)_4N^+OH^-$, $\text{iso-}C_3H_7NH_2$, $(C_2H_5)_2NH$, $(CH_3)_3C\text{-}NH_2$, C_4H_9OH
Stoichiometry		
$M\cdot5\frac{3}{4}H_2O$, $M\cdot7\frac{2}{3}H_2O$	$M\cdot17H_2O$, $M\cdot M'_2\cdot17H_2O$	$M\cdot(5\text{–}40)H_2O$
Unit Cell		
$46H_2O$	$136H_2O$	Variable number of H_2O
Polyhedra	Polyhedra	Polyhedra
2 H_{12}(5 Å)	16 H_{12}(5 Å)	H_8, H_{12}, H_{14}, H_{15}
6 H_{14} (6 Å)	8 H_{16}(7 Å)	H_{16}, H_{17}, H_{18}, H_{60}, *etc.*
Faces	Faces	Faces
Pentagons	Pentagons	Quadrilaterals
Hexagons	Hexagons	Hexagons
		Heptagons
$M_2\cdot M_6\cdot46H_2O$	$M_8\cdot M'_{16}\cdot136H_2O$	

[a] Reprinted, with permission, from Ref. 135, p. 109. (H_n symbolizes a polyhedron with n faces.)

(Table I). Water clathrates consisting of 46 water molecules associated with eight guest molecules in each unit cell are in the first classification. The unit cell has eight cavities; two are dodecahedrons with a diameter of 5 Å, while the remaining six are tetrakaidecahedrons with a diameter of 6 Å (Fig. 4). As indicated in Table I, many small molecules can form clathrates with water. If only the larger cavities are occupied, the stoichiometry is $M_6\cdot46H_2O$ (where M is an inert gas molecule); however, mixed hydrates are possible, with the stoichiometric formula of $M_2\cdot M_6\cdot46H_2O$ (135).

The second class of water clathrates forms with larger guest molecules (Table I). The unit cell consists of 136 water molecules oriented as dodecahedra enclosing 24 interstitial spaces, 16 5 Å in diameter and eight 7 Å in diameter (Fig. 5). The molecules listed in Table I would occupy the larger cavities, although mixed hydrates form with smaller molecules occupying the 16 smaller cavities. In the third class of water hydrates, the cage may be formed of portions of the simpler polyhedra (136, 137). There is no specified unit cell to describe the third class of clathrates, because each guest molecule requires a specific surrounding cage.

The water clathrates are stabilized by the energy of the hydrogen bonds of the water framework, al-

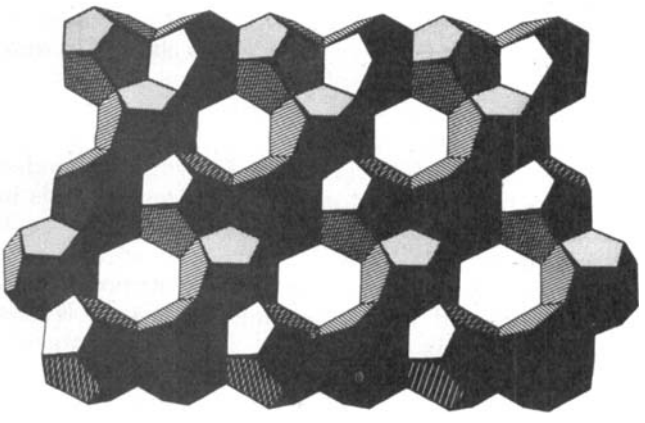

Figure 4—*Structure of the type I hydrate. The tetrakaidecahedral cavities (12 pentagonal plus two hexagonal faces) are formed when layers of dodecahedra, arranged as shown, are stacked on top of each other so that the hexagonal openings line up perpendicular to the plane of the drawing. (Reprinted, with permission, from J. F. Catchpool, in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Eds., Freeman, San Francisco, Calif., 1968, p. 345.)*

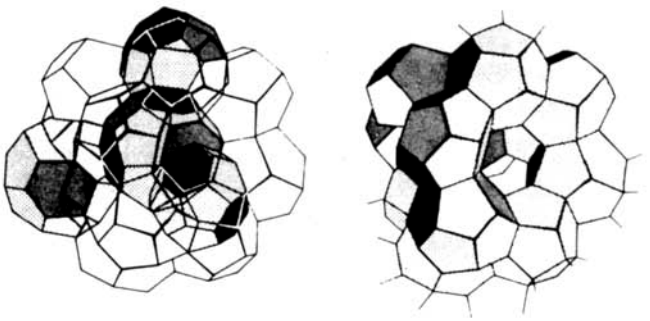

Figure 5—*Two drawings of a folded-paper model of the hexakaidecahedral cavity of the type II hydrate structure. The 16-sided cavities are formed between 16 dodecahedra framing four hexagonal faces. The plane of each hexagonal face lies on the side of a regular tetrahedron. Each hexagonal face is shared by two hexakaidecahedra. (Reprinted, with permission, from J. F. Catchpool, in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Eds., Freeman, San Francisco, Calif., 1968, p. 345.)*

though the hydrogen bond stabilization of the clathrate is less than that in ice due to the more open clathrate structure (138). Van der Waals interactions between the guest and host molecules contribute, however, to the high degree of the overall stability of the clathrate.

A statistical mechanical model for several gas hydrates based on the Lennard–Jones and Devonshire model has been described (139) and the dissociation pressures, compositions, and heats of dissociation were calculated. Daee *et al.* (140) determined that the Helmholtz free energy of formation of the water clathrate was not a smoothly increasing function of the number of molecules but instead showed a minimum corresponding to closed cages. Phase diagrams for several water clathrate systems have been reported (141–143). Calculations of water vapor pressure over a crystalline gas hydrate indicated the formation of the clathrate structure (144). Phase equilibrium studies indicated the existence of mixed hydrates and of multiple hydrates where more than one guest can occupy a cavity (145). Multiple occupancy is limited to small guest molecules such as neon.

The stable clathrate crystals form when water and a gas or volatile liquid are brought together under high pressures and low temperatures. This process can be used, under proper conditions, for the separation of gas (146) or liquid (147) mixtures. Argon can be separated from krypton, krypton from xenon, and short chain (C_3 and C_4) aliphatic olefinic hydrocarbons from mixtures of higher molecular weight compounds. One interesting and novel approach to the desalination of water depends upon the formation of a propane or halogenated propane clathrate in sea water under reduced pressure and temperature (148). The water clathrate that forms can be separated from the salt water and decomposed by heating to 7.2° (45° F) under pressure to give propane and salt-free water. However, water clathrates do not form in highly concentrated salt solutions (149). The melting temperature of clathrates with a high proportion of empty cavities decreases with increasing pressure while the reverse holds for clathrates with a small proportion of empty cavities (150).

Clathrates are formed not only by inert gases but by hydrocarbons and their derivatives (151, 152), inorganic gases, and a number of polar compounds (Table I). Some guests interact with, or are joined to, the water lattice while others rotate freely (153, 154). Pauling (138) proposed an intriguing mechanism for the action of anesthetic gases based upon the formation of minute water clathrate crystals in the brain. He suggested that the anesthetic actions of certain gases of low chemical reactivity (*e.g.*, chloroform and cyclopropane) may result from clathrate formation in the encephalonic fluid. According to this theory, the clathrates would trap ions and electrically charged side chains of protein molecules in such a way as to decrease the energy of electric oscillations in the brain. (Figure 6 shows a diagram of a similar system.) This process would tend to produce a loss of consciousness due to increased electrical impedence of the neural network to electrical waves.

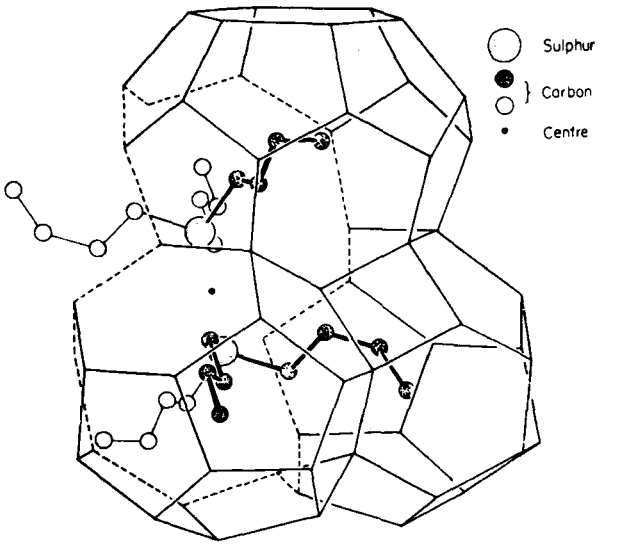

Figure 6—*Projection of clathrate cavity containing a pair of $(C_4H_9)_3S^+$ ions. Only one-half of the cavity is shown, that into which the shaded carbon chains project. The second half surrounds the open-circle carbon chains. The complete polyhedron is constituted of 60 faces. (Reprinted, with permission, from Ref. 135, p. 111.)*

In support of his theory, Pauling determined that a correlation exists between the narcotizing partial pressure of the anesthetic gases and the partial pressure necessary to cause the formation of hydrate crystals. As Pauling indicated, however, approximately the same correlation would be found between the anesthetizing partial pressure of the nonhydrogen-bonding anesthetic agents and any property based upon the van der Waals attraction of the agents for other molecules, *e.g.*, the lipid solubility of the anesthetic gases or the oil–water partition coefficient. The energy of the intermolecular interactions is nearly proportional to the molecular polarizability of the anesthetic agents. If water clathrates do indeed form in the brain and do have a biological function, then it would be a logical extension of this phenomenon to expect their occurrence in other body fluids. This interesting prospect is worthy of further investigation.

Abundant information attests to the formation of hydrate "icebergs" around many ions and proteins in aqueous systems. Included in this category are the trialkylsulfonium and tetraalkylammonium salts. Although the trialkylsulfonium salts and tetraalkylammonium salts are univalent electrolytes, they show a behavior similar to that of the water clathrates (11, 155). Typical formulas for each of these compounds are $2(n\text{-}C_4H_9)_3S^+F^-\cdot 40H_2O$ and $2(\text{iso-}C_5H_{11})_4N^+F^-\cdot 76H_2O$, respectively.

Dyadin *et al.* studied the influence of cation size on clathrate formation in water–quaternary ammonium salt systems (156) and determined the phase diagram for the water–tetra-*n*-butylammonium bromide system (157). Wen (158) reviewed the formation of clathrates of tetraalkylammonium salts.

The crystal lattice of the tri-*n*-butylsulfonium compound resembles the Class I water clathrate with

46 water molecules in the unit cell, except that six of the water molecules are replaced by the two sulfur ions and by the two fluorine ions. The remaining 40 water molecules form the clathrate framework. The interstitial cavities are occupied by the six *n*-butyl hydrocarbon chains rather than by gas molecules. The tri-*n*-butylsulfonium compound is considered to be structurally similar to the chlorine hydrate. McMullan and Jeffrey (155) established the structural arrangement of tetraalkylammonium salts to be similar to the water clathrates. An interesting aspect of the clathrate is that it is highly stable. Although the inclusion complex is 68% water, it remains stable as an ice up to 31.1° (88° F).

The proteins are also highly hydrated crystals with thousands of water molecules per molecule of protein, comprising as much as 90% of the crystal; as such, a water clathrate structure may be expected to exist around the protein molecule. The presence or absence of the water clathrate surrounding the protein molecules may influence the biological function (or lack of function) of the protein. In addition, protein molecules themselves appear to resemble host compounds in that the disulfide bridges between protein layers create spaces between the layers that are capable of enclosing certain hydrocarbons (159).

Phenol—Investigation of the solubility of components of petroleum in liquid hydrogen sulfide led to the discovery of the phenol–hydrogen sulfide clathrate (160). Phenol forms a clathrate with various compounds, including xenon; hydrogen selenide, chloride, bromide, iodide, and sulfide; carbon dioxide, carbon disulfide, and carbon disulfide plus air; carbonyl sulfide; methyl bromide; methylene chloride; tetrafluoroethylene; and 1,1-difluoroethane (161, 162). Lahr and Williams (163) also reported the formation of phenol clathrates with argon and krypton. Some derivatives of phenol also formed clathrates (12, 164). The phenol clathrates were prepared by dissolving phenol in solutions of the guest component or, in the case of gases, dissolution of the gas under pressure. The phenol clathrates separated readily from the solution and were stable at room temperature, but there may be a slight diffusional loss of gas with time.

As described by Mandelcorn (9), the structure of the phenol clathrate depends upon hydrogen-bonded O—O linkages formed by hydroxy groups of six phenol molecules, the linked molecules forming a planar hexagon with the phenyl nuclei oriented alternately above and below the hexagon. A cage is formed when a pair of the six-membered groups are oriented with the hexagons opposite and parallel to one another. The unit cell is rhombohedral, with each corner occupied by the cage arrangement of the 12 phenol molecules, giving an elongated, rather large cage. The unit cell of the phenol clathrate consists of 12 phenol molecules, one large cavity, and a small cavity. Mandelcorn also described the maximum stoichiometry of the phenol clathrates as a function of the size of the guest component.

Dianin's Compound—Dianin's compound (4-*p*-hydroxyphenyl-2,2,4-trimethylchroman) is the condensation product of two molecules of phenol and one of mesityl oxide; upon alkaline fusion, it gives salicylic acid as a product. Dianin (165) observed that this compound retained fixed amounts of the solvent from which it was recrystallized. Several studies showed that Dianin's compound formed clathrates with sulfur dioxide, iodine, argon, and nearly 40 other organic compounds (166, 167). Other guest compounds include heptanol (168), di-*tert*-butyl nitroxide (169), and carbohydrates (170).

A complex structure was proposed in which six molecules of Dianin's compound formed a clathrate cavity (166). The structure has been described as being similar in shape to an hourglass from which the globes have been cut off in the middle. A hexagon of hydrogen-bonded hydroxy groups forms the constriction at the midpoint, and six molecules of Dianin's compound alternately point up or down to form the cups above and below the planar hexagons. These structures are stacked linearly upon each other in the crystal with their hexagons parallel to and above each other. The open end of two cups face each other to form a cavity when the adjacent molecules are held together by strong van der Waals attractions.

Detailed structural analysis by X-ray diffraction has been reported for crystalline inclusion compounds of Dianin's compound with ethanol or chloroform (171). Only one hydroxy group in Dianin's compound is available for hydrogen bonding, so an infinite network of cages does not occur. However, the stacking arrangement in the crystal gives separate columns of individual cages. The hourglass-shaped cage of Dianin's compound is large, approximately 11 Å long and 6.2 Å at its point of maximum width, or about twice as long as the hydroquinone cage.

Mandelcorn (9) compiled a list of the three maximum composition formulas for the Dianin's compound clathrates, depending upon the size of the guest component. Although there are no reported pharmaceutical uses of Dianin's compound clathrates, many simple synthetic precursors and solvents form complexes with Dianin's compound, suggesting that these inclusion compounds might be useful in drug synthesis or purification (172).

Cycloveratril—Formaldehyde and 1,2-dimethoxybenzene (veratrole) combine to form cycloveratril (Fig. 7), which forms numerous clathrates with organic compounds. Caglioti *et al.* (173) prepared clathrates of cycloveratril and benzene, chlorobenzene, chloroform, toluene, acetone, thiophene, carbon disulfide, butyric acid, acetic acid, and decalin. These workers proposed that the benzene rings of the cycloveratril molecule are tilted, giving a nonplanar configuration to the molecule. Strong repulsions between hydrogen atoms of adjacent benzene rings, through rotations of the aliphatic to aromatic carbons, are responsible for the tilting of the benzene rings. Packing of the nonplanar molecules in the crystal leaves small cavities in which guests of small molecular volume can be accommodated.

Miscellaneous Compounds—Over 75 years ago, Hofmann and Küspert (174) reported that a crystalline precipitate formed when benzene was added to a

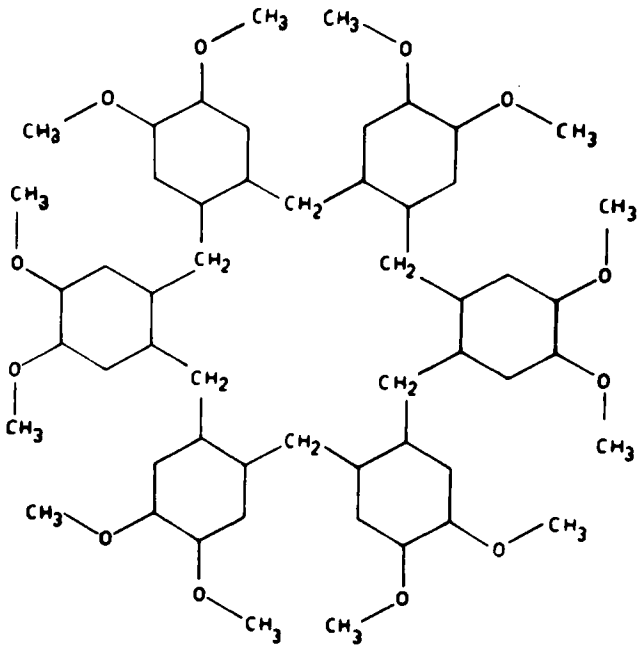

Figure 7—*Structural formula of cycloveratril. (Reprinted, with permission from Pergamon Press, from Ref. 173.)*

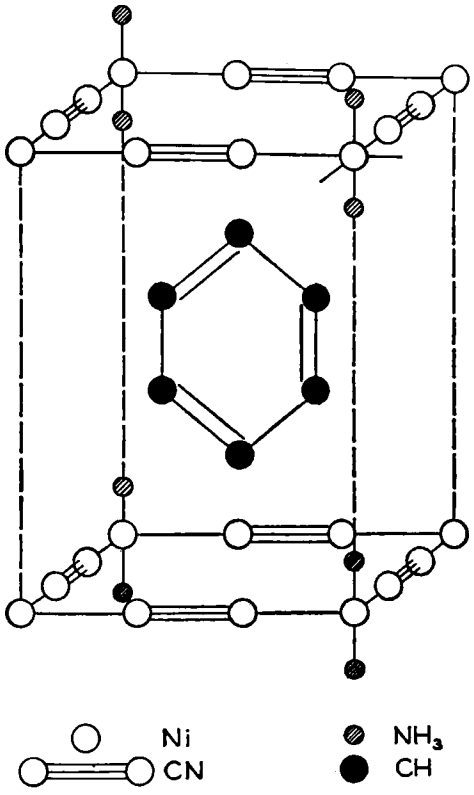

Figure 8—*Schematic representation of the clathrate compound, $Ni(CN)_2NH_3$–C_6H_6. (Reprinted, with permission, from Ref. 175.)*

solution of nickel cyanide in aqueous ammonia solution containing acetic acid. This report was the first on the formation of a clathrate of an organic molecule in an inorganic cage. Numerous organic compounds form clathrates with the nickel cyanide–ammonia complex (12), whose maximum composition formula is $Ni(NH_3)^{+2}2CN^-$·M, where M is the guest molecule. The guest molecules are enclosed in a cavity formed horizontally by two planes of nickel cyanide and vertically by parallel pairs of ammonia molecules. The ammonia molecules are bonded to the nickel atoms and project above and below each plane, giving rise to the cavity when the complex crystallizes (175) (Fig. 8).

The nickel cyanide–ammonia clathrates and similar clathrates are used for the separation of benzene from hydrocarbon mixtures by selective clathration (176). High purity benzene can be recovered from the clathrate under vacuum or by steam decomposition. Clathrates of this type are frequently called "Hofmann's benzene clathrate" if benzene is the guest component or "Hofmann-type clathrates" for other enclathrated guests.

Related to the monoamminonickel (II) cyanide clathrates are clathrates where certain Werner complexes or hexamethylisocyanidoferrous chloride act as the host component. The Werner complexes differ from the nickel cyanide–ammonia complex in that there are usually four neutral molecules and some anions coordinated with the metal ion. An example of a Werner complex forming clathrate compounds is tetra(4-methylpyridine)nickel (II) dithiocyanate. A number of transition elements forming Werner complexes also form clathrates including cobalt, manganese, iron, copper, and zinc. The basic nitrogen compound and the anion also may be varied; the latter may be mono- or polyatomic.

The clathrate compounds of Werner complexes are used industrially for separating and resolving organic mixtures (177–179) and for separating aromatic isomers in liquid phase chromatography (180).

The last of the cage-forming compounds is hexamethylisocyanidoferrous chloride. Water is the guest component in the clathrate, and the overall formula is $Fe(CNCH_3)_6^{+2}2Cl^-·3H_2O$. The $CNCH_3$ groups project octahedrally around each ferrous ion, the unit cell being composed of two hexagonal layers of the ferrous ion (181). No other clathrates of this compound are known.

Bhatnagar (12) discussed these clathrates extensively and there are numerous references in the literature.

Compounds Forming Either Channel or Cage Void Spaces—Tri-*o*-thymotide (182), a versatile host molecule, forms clathrate complexes with many organic compounds (25). The clathrates of tri-*o*-thymotide are unique in that they are arranged in trigonal spirals. The spirals can enlarge in all directions without any constraints, unlike those formed in the hydrogen-bonded hydroquinone framework in which an increase in size in one direction causes a decrease in size in the other.

The general formula is $2C_{33}H_{36}O_6$·M, when the guest molecule (M) has a length not exceeding 9.5 Å. However, very small molecules (*e.g.*, carbon dioxide) have a low probability of clathration. The structure of the clathrate is that of an elongated cage. For molecules longer than 9.5 Å, a channel-like structure is produced and the formula becomes $C_{33}H_{36}O_6$·*x*M,

where x decreases as the chain length of the guest increases.

The tri-*o*-thymotide clathrates are stable; certain complexes can be heated to 100° above the boiling point of the guest compound without loss of the guest. In addition, Lawton and Powell (25) observed spontaneous optical resolution with tri-*o*-thymotide clathrates. If tri-*o*-thymotide is crystallized from a racemic solvent, the cavities of any one crystal will enclose either the dextro- or levo-form of the solvent molecule. If the two different crystals are separated, the solvent can be likewise separated.

MONOMOLECULAR INCLUSION COMPOUNDS

By definition, monomolecular inclusion compounds represent the complexation of a single host molecule and a single guest molecule. The host molecule is characterized by the presence of a cavity or hole into which the guest molecule is inserted.

Cyclodextrins—The most thoroughly investigated monomolecular inclusion compounds are those formed by the cyclodextrins, which are also known as cycloamyloses and as Schardinger dextrins.

The cyclodextrins are water-soluble, nonreducing, macrocyclic polymers, containing glucose molecules joined by α-1,4-linkages (Fig. 9). The most common of these compounds are the α-, β-, and γ-cyclodextrins formed by six, seven, and eight glucose units, respectively. The ring-shaped molecule encloses a cavity of about 6, 8, and 10 Å in diameter for the α-, β-, and γ-cyclodextrins, respectively.

The cyclodextrins are produced by the degradation and cyclization of starch by an enzyme produced by *Bacillus macerans*. The biosynthesis and properties of the cyclodextrins have been discussed in detail (183–188).

Molecules of a suitable size and shape can be held within the cavity of a particular cyclodextrin by van der Waals forces. Advantage of this property is taken in the selective precipitation of the α-, β-, and γ-cyclodextrins from the crude mixture of cyclodextrins produced by the enzymatic process. Cyclohexane or trichloroethylene forms an insoluble inclusion compound with α-cyclodextrin, fluorobenzene or toluene forms an insoluble inclusion compound with β-cyclodextrin, and bromobenzene or anthracene forms one with the γ-cyclodextrin (184). The cyclodextrins readily form inclusion compounds with a great number of organic compounds, including many derivatives of benzene.

The inclusion compounds formed with apolar molecules are only slightly soluble in water (184), whereas those formed with polar molecules are moderately soluble (189, 190). As indicated by Lammers *et al.* (191), the structures of the cyclodextrin inclusion compound in solution and in the crystal differ significantly. In solution, the guest molecule occupies the cavity in the cyclodextrin host and the entire complex is surrounded and solvated by water molecules. In the crystal, however, the guest may be enclosed in a void space of a lattice and not necessarily by individual cyclodextrin molecules. This arrangement may result in the formation of nonstoichiometric inclusion compounds. Exceptions to this behavior are certain cyclodextrin derivatives that do not form lattice-structured inclusion compounds, *e.g.*, cyclodextrin–epichlorohydrin resins (192).

The cyclodextrin cavity was described in the earlier literature as being hydrocarbon in nature, based upon the assumption of a boat configuration of the glucose monomers. Later NMR and X-ray studies confirmed the C-1 chair conformation for the glucose molecules (193). The cyclodextrin molecule would then be arranged with primary and secondary hydroxy groups around the opening of the cavity on opposite ends of the torus with H-3, H-5, and H-6 located within the cavity and H-1, H-2, and H-4 located on the exterior. Cramer and Dietsche (194) proposed that the interior of the cavity behaves as a Lewis base due to its high electron density. Broser and Lautsch (195) suggested that the cyclodextrin was electrophilic due to the increased stability of an inclusion compound in solution with increased basicity of the guest component.

Inclusion of a guest molecule within the cyclodextrin cavity can be utilized to increase the solubility or stability of the guest species. As indicated earlier, many organic compounds form inclusion compounds with the cyclodextrins, including various molecules of pharmaceutical interest. Vitamin A and several unsaturated fatty acids have been stabilized by cyclodextrin inclusion, protecting them from exposure to oxygen and thereby retarding oxidation (88). Cohen and Lach (196) found that α- and β-cyclodextrins formed complexes with the hydroxybenzoic acids and *p*-hydroxybenzoates. Hydrogen bonding was determined to play a role in the formation of the complex, particularly with the hydroxybenzoic acid complex in aqueous solution.

These studies were later extended to include various other drug molecules including sulfadiazine, tetracycline, morphine, aspirin, benzocaine, ephedrine, and sorbic acid (197). In general, the smallest drug molecules showed the greatest complexing activity with the cyclodextrins, and those compounds with the lowest water solubility showed the greatest percent increase in solubility as a function of the concentration of cyclodextrin. Most compounds investigated were too large to fit in the cyclodextrin cavity, leading to the conclusion that the observed interaction was a combination of attractive forces (hydrogen bonding) and inclusion formation.

Pauli and Lach (198) reported that unsaturated phenyl-substituted carboxylic acids were less reactive with β-cyclodextrin than were the corresponding saturated acids. As the chain length of the saturated acids increased, giving greater separation of the phenyl ring and the carboxyl group, the interaction became stronger.

A large number of drug molecules such as reserpine, cortisone acetate, adiphenine, tripelennamine, and testosterone form inclusion compounds with β-cyclodextrin, even though they are too large to be completely included within the cyclodextrin cavity (199). The smaller the guest molecule, the greater is

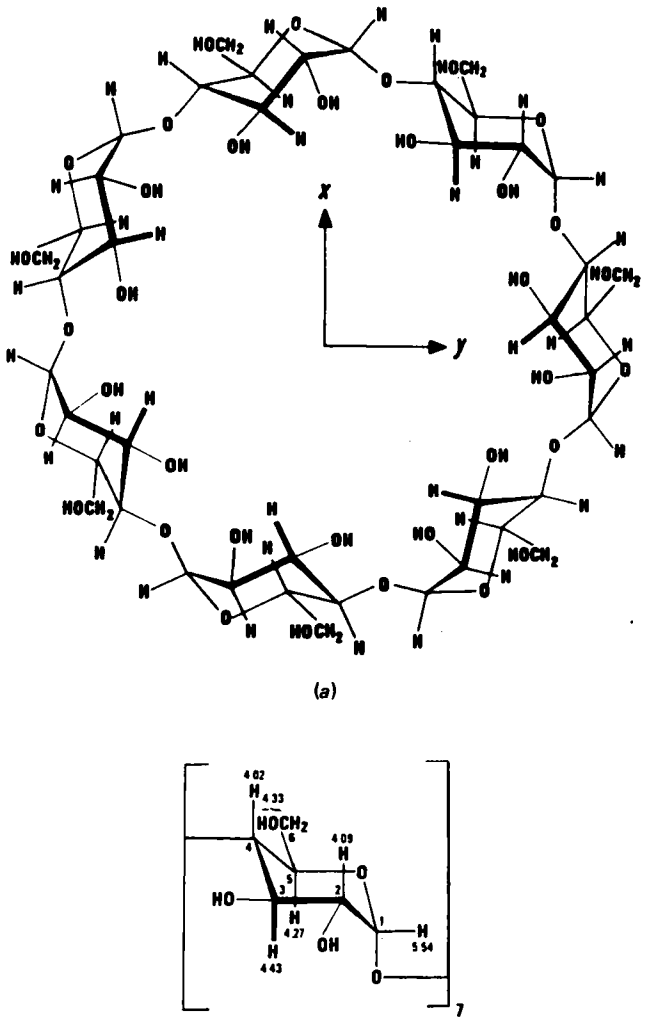

Figure 9—*(a) Cycloheptaamylose molecule. The z-axis is parallel to the cycloheptaamylose cavity, i.e., normal to plane of the page. (b) Recorded chemical shifts in parts per million for various cycloheptaamylose protons. (Reprinted, with permission, from Ref. 203.)*

its complexing activity. Likewise, the degree of activity for the large molecules is dependent on the presence of a suitable group or ring capable of entering the cavity. The ratio of the drug to β-cyclodextrin in the inclusion compounds was 1:1 (adiphenine and tripelennamine) or 2:1 (cortisone acetate and testosterone). The formation constants were relatively high, indicating stable complexes.

The interactions of β-cyclodextrin with barbiturates (200), copper II (201), and several prostaglandins (202) also have been reported. The insoluble inclusion compound of the prostaglandins was collected, dried, and reported to be formulated into suspension, capsule, tablet, suppository, topical, and parenteral dosage forms (202).

Investigations of the apparent formation of inclusion compounds between cyclodextrins and various drug molecules have made significant contributions toward resolving the stoichiometry and mechanism of formation of these complexes. But these studies did not provide conclusive, direct proof of the formation of an inclusion compound. Such evidence has resulted from proton magnetic resonance studies of the inclusion compounds formed between β-cyclodextrin and substituted benzoic acids, aspirin, tetracycline, *d*-phenylalanine, substituted phenols, and other compounds (203). Protons H-3 and H-5, located in the interior of the β-cyclodextrin torus, were shielded by guest protons upon the formation of an inclusion compound; increasingly larger chemical shifts of H-3 and H-5 were detected with increasing concentrations of *p*-hydroxybenzoic acid up to saturation concentration. This study and a subsequent study (204) demonstrated that large molecules can form inclusion compounds if an aromatic moiety or other group of a suitable size and shape can fit into the β-cyclodextrin cavity.

Thakkar *et al.* (205) reported correlation between the formation constants measured by phase solubility analysis for β-cyclodextrin–barbiturate complexes with those obtained by quantitative treatment of induced Cotton effects in the circular dichroism spectra of these inclusion compounds. Circular dichroism was used earlier to study the interaction of α- and β-cyclodextrins with azo dyes (206) and similar complexes with methyl orange and benzoic acid (207).

Cramer *et al.* (208) described the formation of α-cyclodextrin inclusion compounds with azo dyes as consisting of six steps:

1. Approach of the guest or substrate molecule to the cyclodextrin molecule.
2. Loss of the water structure within the cyclodextrin cavity with removal of some water molecules.
3. Breakdown of the water structure around the portion of the substrate that will be included and transport of some water molecules into the solution.
4. Interaction of substituent groups of the substrate with groups on the rim or inside the cyclodextrin ring.
5. Possible formation of hydrogen bonds between the substrate and the cyclodextrin.
6. Reestablishment of the water structure around the exposed parts of the substrate after inclusion has occurred.

These authors (208) pointed out that the rate of formation of the complex should be dependent on the steric factors of Steps 1, 4, and 5. Within the same class of guest molecules, Steps 1, 2, and 6 should not be rate determining. If kinetic specificity with regard to substituent groups is found, Step 3 or 4 and, possibly, Step 5 must be considered. The influence of the breakdown of the water structure and the removal of water molecules from within the ring must also be considered, particularly for the azo dyes. It was determined that the rates of recombination of nitrophenol, its anion, and an azo dye were diffusion controlled; Step 1 was the rate-determining step.

Hoffman and Bock (209) studied the interaction of cyclodextrins with nucleic acids. They determined that only adenosine and inosine compounds (adenosine monophosphate and inosine monophosphate) interacted with β-cyclodextrin and that none interacted with α-cyclodextrin. The primary factors determining the formation of the inclusion complex in ad-

dition to the degree and position of phosphorylation, pH, and polymerization of the nucleotide were the size of the base and its ability to be accommodated in the cyclodextrin cavity. Bases in single-stranded stacked conformation interact to form a complex. Formoso (210) reported the binding constant of 5′-adenosine monophosphate to β-cyclodextrin as well as the interaction of β-cyclodextrin with other nucleic acid monomer units. Hydrophobic interactions did not apparently contribute significantly to the stability of the complexes.

The spatial requirements for the formation of a cyclodextrin inclusion compound in part mimic the "lock and key" mechanism of enzyme catalysis which occurs if the substrate or guest molecule is oriented properly with respect to the active centers of the host (211). It is also possible to use the cyclodextrin ring to include or hold a molecule partially, blocking some reactive sites on the guest but exposing others. Breslow and Campbell (212) produced *p*-chloroanisole by forming the α-cyclodextrin–anisole inclusion compound in which the hydroxy groups rimming the cyclodextrin cavity were converted to intracomplex hypochlorite groups by reaction with hypochlorous acid. The hypochlorite groups react catalytically to chlorinate the exposed *para*-position of the partially included anisole molecule.

Lach and Chin (213) reported the inhibitory effect of β-cyclodextrin on the hydrolysis of ethyl *p*-aminobenzoate (benzocaine). They attributed this effect to the complete inclusion of the ester in the β-cyclodextrin cavity, protecting the ester linkage from attack. Their results indicated that the degradation of benzocaine was dependent on the uncomplexed drug in solution and not on the total amount present.

The results of this work, however, differed from those reported by Bender *et al.* (214, 215) and Van Etten *et al.* (216, 217), which indicated that the cyclodextrins accelerated ester hydrolysis, primarily by nucleophilic attack of the alkoxide ion of the cyclodextrin on the ester within the cyclodextrin cavity. Chin *et al.* (218) explained the apparent difference by observing that the compounds that form an inclusion compound in which the entire molecule (*e.g.*, benzocaine) or the susceptible portion are located in the cyclodextrin cavity (*e.g.*, atropine) exhibited a deceleration in the rate of hydrolysis; the rate of hydrolysis was dependent on the free ester in solution. Conversely, compounds that undergo partial inclusion but leave the active center fixed in close proximity to the hydroxy group of the cyclodextrin (*e.g.*, aspirin) underwent acceleration of hydrolysis. In this case, the rate of hydrolysis is dependent not only on the free ester in solution from dissociation of the complex but also on the nucleophilic attack of the alkoxide ion of the cyclodextrin on the ester linkage. An ester linkage that could be included within the β-cyclodextrin cavity, but only partially within the α-cyclodextrin cavity, would show deceleration of hydrolysis in the former and acceleration in the latter.

Cramer and Kampe (219) reported catalysis of the decarboxylation of methylphenylcyanoacetic acids with α- and β-cyclodextrins in aqueous solution. The rate of reaction was accelerated up to a factor of 14.8, depending on the size of the substituent groups and the nature of the inclusion compound formed. Additional information concerning the relationship between enzyme and inclusion catalysis was reported by Hennrich and Cramer (220), who studied the stereospecific catalysis of the fission of pyrophosphates by cyclodextrin. Cyclodextrins accelerated the cleavage of symmetrical diesters of pyrophosphate with phenols, which are stable in neutral or alkaline solution. β-Cyclodextrin was the most efficient catalyst and the assumption was made that a single phenyl group entered the cyclodextrin cavity, enclosing the pyrophosphate on one side only. Reaction mechanisms were proposed in which the cyclodextrin functions as a neighboring group and/or acts by a push–pull polarization of the pyrophosphate, the result being that the internal phosphate residue of the guest is transferred to the cyclodextrin host.

The cyclodextrins have been used to carry out many other stereospecific catalyses (194, 211). In one case, α-hydroxy ketones were converted from their keto to enol forms upon inclusion in the α-cyclodextrin cavity. The cyclodextrin catalyzed the reaction, which occurs only when the compound is in the enol form.

Cramer and Mackensen (221) synthesized amorphous, nonstoichiometric cyclodextrin–imidazole compounds by reacting cyclodextrins and cyclodextrin derivatives with 4(5)-chloromethylimidazole or 4(5)-aminoalkyl imidazoles. These compounds accelerated the hydrolysis of *p*-nitrophenol acetate about 300-fold. The cyclodextrin–imidazole compounds appear to be models for the complexation of a hydroxy group of the amino acid serine and an imidazole residue of histidine in the active site of chymotrypsin.

Solms and Egli (222, 223) prepared insoluble cyclodextrin–epichlorohydrin resins from mixed cyclodextrins. These compounds were useful in gel chromatographic separations of *o*- and *p*-nitrophenol and of phenylalanine and tryptophan. Wiedenhof and coworkers (192, 224, 225) prepared the α- and β-cyclodextrin–epichlorohydrin resins and determined that they have affinity for aromatic compounds. The isothermal inclusion of undissociated benzoic acid could be described by a Langmuir isotherm; however, the inclusion of *m*-chlorobenzoic acid could be described only by a Freundlich isotherm. Lammers *et al.* (191) also prepared soluble epichlorohydrin resins of α- and β-cyclodextrins. The water-soluble derivatives of α- and β-cyclodextrins form soluble inclusion compounds with nonpolar guest molecules, in contrast to the very slightly soluble complexes the same guests form with the cyclodextrins themselves. These derivatives should prove useful in studying the nature of the cyclodextrin inclusion compounds in solution.

Hoffman (226) separated various nucleotide and nucleoside mixtures, particularly those containing adenine, on stable, cross-linked β-cyclodextrin gels, similar to the mixed cyclodextrin gels described by Solms and Egli (222). The column chromatographic separations were believed to occur by the formation of an inclusion compound. A process for using cyclo-

dextrin gels was patented for the separation of vitamins, perfumes, and aromatic amino acids (227).

The partial resolution of racemic mixtures has been accomplished with modest success by selective inclusion with α- and β-cyclodextrins. β-Cyclodextrin has been particularly effective in differentiating between *d*- and *l*-forms; Cramer (83) partially resolved a number of esters using this host molecule. The resulting resolutions ranged from 0.84 to 11.33%. Mikolajczyk *et al.* (228) partially resolved sulfoxides with β-cyclodextrin, achieving resolutions ranging from 4.4 to 14.5%.

Miscellaneous Compounds—Aside from the cyclodextrins, other substances appear to fit into the classification of monomolecular inclusion compounds. Many large molecules have cavities or other structural arrangements capable of partially or completely enclosing a guest molecule (6). Examples of such compounds are the bis-*N,N'*-alkylenebenzidines which, when crystallized, can enclose a molecule of benzene or of dioxane in an intramolecular ring (229).

Cramer (6) suggested that proteins, minerals, cellulose, and graphite form monomolecular inclusion compounds. The specificity of reactions between various proteins and guest molecules such as those of dyes and other compounds with serum albumin, chromoproteins, and hemoglobin suggests a possible inclusion process; however, normal salt formation could account for this complexation and may negate this concept. Another strong possibility for the consideration of proteins as monomolecular inclusion compounds are the highly specific antigen–antibody interactions, which are dependent upon shape-matching factors. The hapten or nonproteineous portion of the antigen fits closely and specifically within the antibody and actually determines the antigen-to-antibody specificity. Hydrogen bonds and electrostatic attractions play a role in leading to a stable, tightly bound complex, in addition to van der Waals forces resulting from the close spatial proximity of the two interacting species.

Further biological ramifications come from suggestions that deoxyribonucleic acid (DNA) may have a function analogous to that of a host molecule (6). Protamines combine with DNA by fitting into a groove running through the helical macromolecule in a screw line (230). The biological activity of the nucleic acids may therefore be related to an inclusion process. The template function of ribonucleic acid (RNA) may also be a specific inclusion process as well as the interaction of gene mutations with DNA.

The antibiotic gramicidin A has a channel along its helix axis which forms a transmembrane channel through the lipid portion of a membrane when gramicidin A interacts with the membrane (231, 232). Although this does not represent an inclusion process *per se*, the presence of a cavity or channel in this compound and in another antibiotic, valinomycin (233), suggests that these compounds may act as host structures. A specific inclusion possibly could occur that might block the transmembrane channel or otherwise alter the biological activity of these compounds, perhaps leading to the synthesis of molecules with channels specific for a particular guest. The concept of inclusion compound formation as a component of the complex biochemistry of the living organism is exciting and should open new vistas in research for a complete understanding of those vital processes in health and disease.

PRODUCTS OF THE BLUE-IODINE REACTION

The blue-black color resulting from the reaction between iodine and starch has intrigued scientists for over a century and a half. In 1939, Freudenberg *et al.* (234) proposed that this behavior was the result of inclusion compound formation. Subsequent work by Cramer (6, 235, 236) indicated that a soluble, channel-like inclusion compound was formed by the polymerization of iodine atoms into a linear "template" around which the starch molecules formed a helix. This type of helical structure enclosing the iodine polymer was stable only as the inclusion compound. Instead of the characteristic red-brown color of iodine solutions, the inclusion compound was blue-black; hence the common name for this phenomenon is the "blue-iodine reaction."

The blue-black color of the cyclodextrin–iodine inclusion compound occurs by a similar process to that of starch (6). When the cyclodextrin–iodine complex crystallizes from solution, the individual cyclodextrin molecules align themselves to form a long, channel-like structure with the cyclodextrin rings lying side by side and the polyiodide chain occupying the now continuous channel. One turn of the starch helix contains six glucose molecules, the same as that of α-cyclodextrin. Both α- and β-cyclodextrins form inclusion compounds with iodine; α-cyclodextrin can also complex with chlorine and bromine. However, the β-compound can complex only with bromine, because the chlorine molecule is too small to stay in the cavity (6). γ-Cyclodextrin forms a loose complex only with iodine.

The linear iodine polymers are also stabilized by inclusion compound formation with many other compounds including benzonitrile, coumarins, cholic acid, cortisone, flavones, benzamide, barbituric acid, quinine and other alkaloids, and benzophenone (6).

The most likely mechanism to explain the nature of the polyiodide chain is that the host molecule acts as an electron donor, forming a loose charge-transfer complex with the iodine (237, 238) (Scheme I). This mechanism is substantiated by the fact that all host molecules forming blue-iodine compounds have such donor groups, *e.g.*, the electron pairs of the oxygen atoms of the cyclodextrins and the carbonyl groups of the flavones and coumarins.

$$\text{donor} + I_2 \rightleftharpoons (\text{donor}, I)^+ + I^-$$

Scheme I

Two other examples of the blue-iodine reaction should be mentioned. The first example is that of swollen cellulose which interacts with iodine to give a blue-black color (6), leading to the concept that the process of dyeing cellulose and other materials is an inclusion process. The second example is the polariz-

ing filter (239). These filters are prepared by treating a sheet of polyvinyl alcohol polymer with iodine, followed by stretching the sheet to orient the linear polyiodide chains within the polyvinyl alcohol hosts. The electrons along the polyiodide chain resemble those of a metal because they can move along the chain. However, they cannot move perpendicular to it, accounting for the very high extinction coefficient of the polyiodide chains in the inclusion compounds. This property of the electrons of the chain allows the absorbance of light polarized parallel to the direction of the stretched orientation of the polyvinyl alcohol–iodine complex, and only the light polarized at right angles passes through. Bolewski and Uchman (240) reported that the clathrating ability of polyvinyl alcohol decreased with an increase in the degree of branching.

MACROMOLECULAR INCLUSION COMPOUNDS

The last category of inclusion compounds includes those of a macromolecular size. In many cases the differentiation between monomolecular and macromolecular inclusion compounds is not clearly defined so considerable overlap results. Some large molecules such as certain minerals, cellulose, and proteins can be discussed in either category.

A detailed discussion of the macromolecular inclusion compounds is unfortunately beyond the scope of this review. Well over a 100 or more publications appear annually concerned with the preparation, properties, and applications of these useful compounds.

Zeolites—The major category of macromolecular inclusion compounds is commonly known as molecular sieves which are widely used in pharmaceutical processes and other chemical and biological applications due to their unique ability to fractionate molecules according to size. Molecular sieves are three-dimensional inorganic crystalline lattices with a myriad of interconnected cavities, capable of retaining or sorbing water or other molecules, depending on the size of the channels and cavities within the crystal. Of the molecular sieves, the most widely used are the many zeolites which occur naturally and are also synthesized for specific purposes.

The zeolites are hydrated aluminum silicates and may contain sodium, potassium, calcium, and barium (241–244). The void spaces of the zeolites normally contain water, but the water can be removed by heating to give a uniformly porous lattice with empty spaces which can be refilled with gases, vapors, or molecules from solution. Although the zeolites give off water vapor when heated, unlike most other water-bearing crystals, they do not then collapse or disintegrate. After cooling, the lost water vapor is replaced or another gas is adsorbed.

X-ray diffraction studies have indicated that the zeolites contain a precise arrangement of cavities linked by channels (242). Water and exchangeable ions can pass through the interconnecting channels and occupy the cavities, facilitating the reversible processes of water removal and ion exchange. The structure of any zeolite crystal is based on a tetrahedral arrangement of oxygen atoms around a silicon or aluminum ion. Barrer and Meier (241) and Barrer and Stuart (242) described the structure of the common zeolite, chabazite, as consisting of two tight hexagons, each with six silicon and aluminum atoms, along with their associated oxygen atoms, facing each other and forming a flat prism. Eight such prisms are joined together to form an oval cavity of approximately 11.0 Å. The cavities are linked by six channels about 3.9 Å in diameter. Twenty-four water molecules and four calcium ions can occupy each chabazite cavity, of which there are 5×10^{21} cavities/in.^{3} of crystal (245). By contrast, the feldspars have the same silicon–oxygen and aluminum–oxygen tetrahedra, but they are arranged in a way that does not allow free spaces capable of enclosing guest molecules.

The zeolites are capable of sorbing, separating, or exchanging similar molecules (243–245). The molecule to be sorbed or exchanged must have a shortest dimension less than the shortest distance between chains in the zeolite lattice. The zeolites sorb only those molecules that are small enough to enter the channels of the dehydrated mineral lattice. Kipling (246) described the selective separation of mixtures of various organic compounds strictly by molecular size. For example, a particular molecular sieve adsorbs hexane while rejecting benzene, but the larger pore size molecular sieves accept benzene. Mixtures of compounds of a suitable size can be sorbed simultaneously; if the zeolite is sequentially exposed to the individual components, the first substance sorbed will be difficult to displace, resulting in very low sorption of a subsequent component (247).

The zeolites are also capable of ion exchange (243, 244); zeolites containing sodium exchange cations with a solution containing calcium ions, a property utilized commercially in the removal of calcium from hard water. The structure of the zeolites is essentially a silicate tetrahedra with partial replacement by aluminum, requiring additional exchangeable cations for electrical neutrality.

Benson and King (248) indicated that the molecular discrimination in adsorption by zeolites is largely due to differences in the polarizability of the adsorbate. Large molecules would be expected to undergo weak adsorption due to their bulkiness, which prevents strong interaction with adsorption sites. There appears to be a good correlation between the strength of adsorption and the polarizability of the adsorbate. Electrostatic bonding rather than dispersion forces is indicated as the mechanism of bonding by the function of certain zeolites which can discriminate between *ortho*- and *para*-hydrogen. Adsorption isotherms are approximately Langmuir in type, and multilayer adsorption does not generally occur above the critical temperature of the adsorbate. Below the critical temperature, multilayer adsorption generally occurs, and capillary condensation may result from the pores or channels limiting the extent of multilayer formation.

Synthetic zeolites can be prepared for specific separations by crystallization under controlled condi-

tions of temperature and the presence of specific ions to determine the channel size (243, 245, 249, 250). Adjustment of temperature affects the vibrations of the atoms in the zeolite crystal, which, in turn, affect the effective diameter of the channels. The essential criterion in the function of these molecular sieves is that the guest molecule be able to pass through the channel. An example of a synthetic zeolite is a sodium-bearing zeolite that will not sieve *n*-octane from isooctane. In fact, neither compound can enter its channels. However, a calcium-bearing zeolite has channels sufficiently large for only the *n*-octane to enter, giving rise to industrial processes used to improve gasolines. Recently synthesized zeolites specifically adsorb cyclohexane, carbon tetrachloride, *n*-hexane, or water (249).

A major use of the zeolites is in the dehydration of solvents or other materials due to their high affinity for water. The sorption capacity of the zeolites also makes them useful for gas storage, for storage and recovery of radioactive material, as carriers for catalysts in chemical reactions, and for many other applications in adsorption separations, hydrocarbon catalysis, and purifications (249).

Dextrans—Other materials capable of selective inclusion include the modified dextrans and certain support media that are used in column chromatography.

The modified dextrans are cross-linked dextran macromolecules that form a three-dimensional network of polysaccharide chains capable of functioning as a molecular sieve and of fractionating molecules according to size (251). Most modified dextrans are hydrophilic and swell in water or electrolyte solution to form a gel, hence the name "gel filtration" for the fractionating ability of these dextrans. There is also a chemical modification of dextran that is lipophilic and swells in many polar organic solvents as well as in water.

The dextran gel matrix has a porosity determined by the degree of cross-linkage in the polysaccharide network. Molecules larger than the maximum pore size of a particular modified dextran do not enter the matrix, whereas smaller molecules diffuse into the matrix to different degrees, depending on their size. Aqueous mixtures separate as a solution is passed through the gel column, the smallest molecules being the last to be eluted from the column. In addition to their molecular sieve capabilities, the modified dextrans are also used as gel matrixes for ion exchange in a manner similar to that of the zeolites. Other related materials used for affinity chromatography include agarose and the polyacrylamide gels.

Silica Gels—The last group of macromolecular inclusion compounds to be discussed is the silica gels. Like the zeolites and modified dextrans, the silica gels are molecular sieves with a high sorptive capacity. The silica gels are also capable of very high selectivity, perhaps greater than that of the other macromolecular inclusion compounds.

Silica gels are composed of an irregular three-dimensional network of silicon atoms connected by oxygen atoms in the same spatial relationship as the hydrogen-bonded oxygen atoms of water. The silica gels are formed by crystallization of the silicon–oxygen network around a guest molecule. After the inclusion structure has formed, the guest can be removed by heating or degassing. Since the guest acts as a template, the silica gel has strong sorptive properties for that particular molecular species. For example, a silica gel made in the presence of butyl orange as the guest will sorb that molecule to a greater extent than it will adsorb propyl orange (243), although it will accept other molecules of a suitable size and shape. The tailoring of silica gel adsorptive properties is a most unique capability that can be of significant importance when it is necessary to have such a highly specific separation.

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RESEARCH ARTICLES

Metabolism of 8-(Methylthio)cyclic 3′,5′-Adenosine Monophosphate by Rats and Dogs after Oral or Intravenous Dosing and *In Vitro* by Subcellular Preparations of Dog Liver

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Abstract □ 8-(Methylthio-^{14}C or -^{35}S)cyclic 3′,5′-adenosine monophosphate (I) was given intravenously to rats (5 mg/kg) and orally and intravenously to dogs (0.25, 2.5, or 50 mg/kg). Oral doses were absorbed well but slowly. Plasma half-lives in dogs were about 3 hr after oral or intravenous doses of 0.25 or 2.5 mg/kg and ranged from 5 to 12 hr after oral or intravenous doses of 50 mg/kg. Plasma glucose and insulin concentrations in dogs were increased by oral or intravenous doses of the compound. Regardless of the route, excretion of radioactivity by rats and dogs at all doses was chiefly in the urine (74–87% of the dose); the remainder was excreted in the feces or bile. Compound I was rapidly distributed to most tissues of dogs but entered the brain and certain portions of the eye slowly and to a limited extent. Urine and plasma of dogs and urine of rats contained I, 8-(methylthio)adenosine, and at least two other unidentified metabolites. Compound I and cyclic 3′,5′-adenosine monophosphate were metabolized *in vitro* by the soluble fraction of dog liver to form 8-(methylthio)adenosine-5′-monophosphate and adenosine-5′-monophosphate, respectively. These compounds were further converted to 8-(methylthio)adenosine and adenosine, respectively. Compound I was metabolized *in vitro* more slowly than cyclic 3′,5′-adenosine monophosphate.

Keyphrases □ 8-(Methylthio)cyclic 3′,5′-adenosine monophosphate—metabolism, dogs and rats after oral and intravenous doses, *in vitro* metabolism by subcellular dog liver preparations □ Adenosine monophosphate—activity mimicked by 8-(methylthio)cyclic 3′,5′-adenosine monophosphate, metabolism of analog *in vivo* and *in vitro* □ Metabolism—8-(methylthio)cyclic 3′,5′-adenosine monophosphate in dogs and rats after oral and intravenous doses and *in vitro* by subcellular dog liver preparations

Ample documentation now exists that the biological effects of cyclic 3′,5′-adenosine monophosphate can be mimicked by certain of its analogs (1, 2). Such biologically active analogs might exhibit greater po-