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Observed Diffraction Pattern and Proposed Models of Liquid Water

Most models are either incompatible with observed x-ray scattering or insufficiently defined for adequate testing.

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Molecular theory can predict some of the macroscopic properties of monatomic fluids from first principles (*1*). The behavior of associated liquids such as water is so much more complex than that of the fluid noble gases that methods of interpretation are much more crude. In order to understand and perhaps predict the bulk properties of such liquids, it is necessary to make assumptions about the "structure" of these systems, that is, about the average arrangement of molecules with respect to each other, insofar as this is nonrandom. Such a system of postulates and inferences is generally called a model of the liquid structure.

Most of the proposed water models attempt to describe the structure and size of associated units and their variation with temperature. Although no single model explains all the known properties of liquid water, several models which differ significantly at the molecular level have been shown to account more or less satisfactorily for a number of the observed thermodynamic, spectroscopic, and transport properties of the liquid (*2*). Thus, the macroscopic behavior of water alone does not furnish a suitable criterion for the acceptance of a particular model. Perhaps the most direct information about the average molecular configuration in the liquid state can be obtained from the diffraction pattern. Even though a model which scatters x-rays in exactly the same way as liquid water cannot be proved to be unique, this agreement is necessary for the model to be tenable. Many proposed water models are not sufficiently defined at the molecular level to permit computation of x-ray intensity and radial distribution functions, and thus these models cannot be tested with the x-ray data. Our critical examination is therefore confined to models for which radial distribution functions have been, or can be, calculated.

Before we enter into a detailed discussion of proposed models for liquid water, we shall outline what kind of information can be extracted from diffraction data on liquids and shall state the conditions that a model must meet if it is to be tested.

X-ray Scattering and Radial Distribution

Heteratomic liquids. Consider a macroscopically isotropic diffracting object in which the relative positions of the atoms are fixed. The intensity, in electron units, of a coherently scattered wave is given by the Debye equation (*3*)

$$I(s) = \sum_{i=1}^{N} \sum_{j=1}^{N} [f_i(s)\, f_j(s) \sin(sr_{ij})]/sr_{ij} \qquad (1)$$

with summation for both i and j over all N atoms in the system. The scattering variable s is defined as $(4\pi/\lambda)\sin\theta$, where λ is the x-ray wavelength and 2θ is the scattering angle. The atomic form factors $f(s)$ are the Fourier transforms of the radial distribution functions for the electron density in the individual atoms, accessible from calculations at the outset. It is convenient to separate terms in Eq. 1 for $i = j$, which are not dependent on a separation distance r_{ij}. The total intensity corrected for these terms (the reduced intensity function) is

$$I(s) - \sum_{i=1}^{N} f_i^2(s) = \sum_{i=1}^{N} \sum_{j \neq i = 1}^{N} [f_i(s)\, f_j(s) \sin(sr_{ij})]/sr_{ij} \qquad (2)$$

In a real object, and particularly in a fluid, relative atomic positions are not rigidly maintained. We represent by $4\pi r^2 \rho_{\alpha\beta}\,(r)$ a distribution function giving the probability that distinct pairs of atoms of kind α,β are to be found separated by a distance r. The functions $\rho_{\alpha\beta}$ represent the average distribution of pairs over both time and the volume of the sample.

In these terms, the scattered intensity becomes

$$i(s) \equiv I(s)/n - \sum_{i=1}^{m} f_i^2(s) = \sum_{\alpha=1}^{m} \sum_{\beta=1}^{m} f_\alpha(s)\, f_\beta(s) \int_0^\infty [4\pi r^2 \rho_{\alpha\beta}(r) \sin(sr)]/sr\, dr$$

in which a structural unit containing m atoms is visualized as representative of the whole sample, which contains n such units. The term

$$\sum_{i=1}^{m} f_i^2(s)$$

is the independent atomic scattering, and the reduced intensity $i(s)$ is the structurally sensitive part of $I(s)/n$, the measured intensity scaled to one structural unit. In order to apply the Fou-

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rier integral theorem, it is advantageous to add and to subtract from the above expression the double sum

$$\sum_{\alpha=1}^{m} \sum_{\beta=1}^{m} [f_\alpha(s)\, f_\beta(s) \int_0^\infty 4\pi r^2 \rho_0 \sin(sr)]/sr\, dr$$

where ρ_0 is the bulk number density of structural units. The added double sum corresponds to a delta function at $s = 0$ which does not contribute to the observable scattered intensity and may therefore be neglected. The expression for the reduced intensity becomes

$$i(s) = \sum_{\alpha=1}^{m} \sum_{\beta=1}^{m} f_\alpha(s)\, f_\beta(s) \int_0^\infty [4\pi r^2\; [\rho_{\alpha\beta}(r) - \rho_0] \sin(sr)]/sr\, dr \qquad (3)$$

with summation over all distinct pairs in the structural unit.

For a liquid system, the pair density functions $\rho_{\alpha\beta}(r)$ should approach closely the bulk density ρ_0 beyond a critical distance $r = r_c$, which may be taken as a measure of the extent of short-range order in the liquid. If it is assumed that all pair density functions $\rho_{\alpha\beta}(r)$ become precisely equal to ρ_0 at large r, it may be shown that $I(s)$ approaches zero at $s \ll 1/r_c$. In practice, it is found that $I(0)$ is finite. This implies that there are persistent long-range variations of $\rho_{\alpha\beta}(r)$ from ρ_0. For a liquid composed of one well-defined molecular species which need not be monatomic, the small-angle scattering conforms to the equation (*4*)

$$I(0)/nF^2 = <(\nu - <\nu>)^2>/<\nu> \qquad (3a)$$

where ν is the instantaneous and $< \nu >$ is the average number of molecules in a volume whose dimensions are large compared to r_c, F is the number of electrons per molecule, and the averages are over time and the volume of the sample. The critical length r_c is always several times larger than molecular dimensions; for water it is estimated (see below) to be about 8 angstroms. For some exceptional systems (for example, fluids near the critical point) it approaches macroscopic dimensions, and the limiting intensity $I(0)$ is reached only at unobservably small scattering angles.

A particular application of Eq. 3a concerns random equilibrium fluctuations in the local number density in a system containing one kind of molecule, in which the average density is also the most probable one, and only small fluctuations are present. Statistical mechanical considerations (*5*) yield for the relative variance in particle number which appears on the right side of Eq. 3a

$$<(\nu - <\nu>)^2>/<\nu> = kT\rho_0\,\kappa \qquad (3b)$$

where κ is the isothermal compressibility, k is the Boltzmann constant, and T is the absolute temperature. Random equilibrium density fluctuations are, of course, present in any system but may be only one source of such fluctuations.

In the special case in which the scattering system consists of one kind of atom, the double sum (Eq. 3) reduces to one term, and it is possible to obtain the atomic distribution functions, $\rho(r)$ by Fourier transformation. In a similar manner, a distribution function for molecular centers can be obtained from Eq. 3, if the system consists of one kind of molecule, and if the molecules are spherically symmetrical and randomly disposed (*6*). In general, however, the corresponding quantities $\rho_{\alpha\beta}(r)$ are not obtainable individually. It is nevertheless useful to construct a modified distribution function by Fourier transformation, namely

$$D(r) \equiv \sum_{\alpha=1}^{m} \sum_{\beta=1}^{m} D_{\alpha\beta}(r) =$$

$$4\pi r^2\rho_0 + (2r/\pi)\int_0^\infty s\,i(s)\, M(s) \sin(sr)ds \qquad (4)$$

with

$$M(s) = [\sum_{\alpha=1}^{m} f_\alpha(s)]^{-2}$$

for $s \leqslant s_{\max}$, the maximum value of s accessible in scattering experiments, and $M(s) = 0$ otherwise. Introduction of this modification function into Eq. 4 makes the product $f_\alpha(s)\; f_\beta(s)\; M(s)$ nearly independent of s and thus removes from the resulting radial distribution function the average breadth of the distribution of electron density in the atoms. The relation between component x-ray pair distribution functions $D_{\alpha\beta}(r)$ and $4\pi r^2\rho_{\alpha\beta}(r)$ is one of convolution

$$D_{\alpha\beta}(r) = 4\pi r \int_{-\infty}^{\infty} u\rho_{\alpha\beta}(u)\; T_{\alpha\beta}(u - r)du \qquad (4a)$$

with

$$T_{\alpha\beta}(r) = (1/\pi) \int_0^\infty f_\alpha(s)\; f_\beta(s)\; M(s) \cos(sr)ds \qquad (4b)$$

The function $D(r)$, accessible from x-ray scattering data, is thus a kind of linear combination of true radial distribution functions, which are convoluted with the known functions $T_{\alpha\beta}(r)$ given by Eq. 4b. The factor $T_{\alpha\beta}(r)$ may be visualized as a shape function into which a pair distribution function is transformed by the combined effects of the inherent electron distribution and treatment of the x-ray data.

It is not possible to decompose the function $D(r)$ uniquely into components $D_{\alpha\beta}(r)$, which can then be deconvoluted into components $\rho_{\alpha\beta}(r)$, unless, for a liquid containing m different types of atoms, $m(m + 1)/2$ independent diffraction experiments are performed. For water, three different experiments involving different values for the form factors f would be necessary. Such a study, although possible in principle by the use of neutron diffraction data on isotopically substituted waters, has not been undertaken.

Since water molecules interact predominantly with long-range, noncentral forces which are not pairwise additive, it is not possible to compute radial distribution functions for the liquid from the known properties of an isolated water molecule. Thus comparison either with theory or with other experimentally known properties of liquid water would not be possible even if the individual pair distribution functions $4\pi r^2\rho_{\alpha\beta}(r)$, rather than only the x-ray radial distribution function $D(r)$, were known. For water the only recourse open, as for other polyatomic liquids, is to calculate the atom-pair distribution functions from proposed models and then to construct intensity and radial distribution functions which can be compared with those derived from x-ray diffraction data.

Models of liquid structure. A model that is to be tested against diffraction data must have a number of properties and must meet certain conditions in order to be useful, realistic, and tractable in this context:

1) It must specify all interactions, properly weighted, to a distance $r \approx r_c$, where r_c measures the extent of order in the liquid.

2) It must do this in terms of a small number of independent parameters.

3) It must ensure that structurally equivalent atoms have the same distance spectra.

4) The average density of interactions must be consistent with the density of the liquid.

5) The distance spectra of different atoms must be geometrically compatible.

Condition 1 is necessary for the calculation of intensity functions according to Eq. 5. We will show below that $r_c \simeq 8$ angstroms for water at room temperature; thus, a water molecule located at the center of a sphere with a radius of 8 angstroms "sees" approximately $(4/3)\pi 8^3 \rho_0 \simeq 70$ other molecules in positions which are, on the average, not random. The expression for the intensity function will thus contain a similar number of terms, and condition 2 is necessary since a description with such a large number of adjustable parameters is meaningless. Any realistic model must generate a distance spectrum in such a way that radial distribution around any atom is, on the average, the same as that around any atom structurally equivalent to it—hence condition 3. It is, of course, permissible to treat chemically equivalent atoms as structurally nonequivalent. Condition 4 requires no comment, and condition 5 is necessary because the distribution of neighbors about any atom is not independent of the distribution about the neighboring atoms: The placement of one atom clearly excludes other atoms from the volume it occupies; furthermore, any one atom must be simultaneously a first neighbor of, for example, four other atoms (tetrahedral coordination), a second neighbor of perhaps 12 others, and so forth.

The only way known to us of fulfilling these conditions is to assume a certain near-neighbor configuration around one or more origin atoms and to repeat this structural unit according to some specified rule. An approach of this kind involves a space lattice. Unlike the crystalline case, however, repetition of the structural unit is imperfect and therefore is accompanied by rapid, progressive loss of positional correlation between atoms in adjacent "primitive cells." This loss of correlation may be embodied in the model in the form of mean-square variations from average separations which increase rapidly with radial distance, and the distance spectrum characteristic of a particular model can be closely approximated by a uniform distance distribution (continuum) a few molecular radii away from any starting point.

For such a description, Eq. 3 takes the form

$$i(s) = \sum_{i=1}^{m} \sum_{j} [\exp(-b_{ij}s^2) f_i(s) f_j(s) \sin(sr_{ij})]/sr_{ij} + \sum_{\alpha=1}^{m} \sum_{\beta=1}^{m} \left\{ \exp(-b_{c\alpha\beta}s^2) f_\alpha(s) f_\beta(s) 4\pi\rho_0 [sr_{c\alpha\beta} \cos(sr_{c\alpha\beta}) - \sin(sr_{c\alpha\beta})] \right\} / s^3 \quad (5)$$

where the summations are over the structural unit for α, β, and i, and over all atoms in the discrete structure for j. The first double sum in Eq. 5 arises from carrying out the integration in Eq. 3 over the discrete structure, that is, for the range $0 < r \leqslant r_c$, and averaging the Debye equation (Eq. 2) over discrete Gaussian distributions with average separation r_{ij} and mean-square variation $2b_{ij}$. The second double sum in Eq. 5 results from integration over the continuum, that is, for the range of r in which $\rho_{\alpha\beta}(r) = \rho_0$. The boundary between the two regions need not be sharp; in Eq. 5, $r_{c\alpha\beta}$ represents the mean and $2b_{c\alpha\beta}$ the variance of this boundary for the pair correlation function $\rho_{\alpha\beta}$. The second double sum corresponds to scattering from the "hole" in a uniform medium in which the discrete structure is situated. Fourier transformation of the reduced intensity function (Eq. 5) according to Eq. 4 yields the x-ray radial distribution function of the model.

The preceding paragraphs describe a way of constructing a one-phase model in which only short-range correlation exists. There has been much discussion of models of a different sort which possess two or more distinct "phases." In order to test the validity of such a model with x-ray diffraction data, much detail is needed that is usually left unspecified, namely:

6) The detailed average arrangement of molecules within each phase.

7) The relative proportions of the two or more phases.

8) The detailed average arrangement of molecules around the boundaries of a phase region.

9) The average separation and relative orientation of pairs of such regions.

Conditions 8 and 9 need not be given in detail if the regions are large compared with r_c and randomly disposed. The assumption of such large domains, however, has important implications with regard to small-angle scattering.

Liquid water. The x-ray radial distribution functions $D(r)$ for liquid water (*8*) (Fig. 1) contain information about the average number of neighbors at various radial distances around any molecule (*9*) taken as the origin. If the liquid were completely uniform, then $D(r)$ would be equal to $4\pi r^2 \rho_0$ (Eq. 4). However, because of the finite size of a water molecule, $D(r)$ is zero up to about one molecular diameter and approaches $4\pi r^2 \rho_0$ only at distances of some five molecular radii. Between these radial distances the radial distribution function differs appreciably from $4\pi r^2 \rho_0$, and this fact must be ascribed to positional correlation of oxygen atoms, that is, "structure" in liquid water.

The following significant features can be deduced directly from the curves of Fig. 1:

A) There are deviations from a uniform distribution of distances (there is "structure") to about 8 angstroms at room temperature.

B) The first prominent maximum, corresponding to near-neighbor interactions, shifts gradually from 2.82 angstroms at 4°C to 2.94 angstroms at 200°C. These numbers are obtained from a large-scale plot of the tabulated (*10*) functions $G(r) \equiv D(r)/4\pi r^2 \rho_0$.

C) The radial distribution of oxygen atoms in water at 4°C is not significantly different from that in deuterium oxide at the same temperature.

D) The average coordination number in liquid water from 4° to 200°C is approximately constant and slightly larger than four, but the first peak in the radial distribution function cannot be described by a single Gaussian distance distribution.

Points A through C follow directly from Fig. 1, but point D requires some discussion. The calculation of average coordination numbers from the area under a peak of a radial distribution function is never unambiguous. In this case, the lower bound of the first maximum is fairly well resolved, but the upper bound is not. Interactions with second and higher neighbors contribute significantly to the area under the first peak, and proper resolution into individual components is quite uncertain. If we assume that the first maximum in the radial distribution function of liquid water is characteristic of the interaction of one oxygen atom with N oxygen atom neighbors separated by a Gaussian distribution of distances centered at r_0, the peak shape can be calculated. The contribution to the peak centered at r_0 from longer-range distances can be estimated as arising from a uniform distance distribution starting at a distance r_c given by the radius of a sphere of volume $(N + 1)/\rho_0$. Using Eq. 5 and adjusting by least-squares the distance r_0 and the mean-square variations in both r_0 and r_c, we found that a value of $N = 4.4$ gives the best agreement with the experimental curves for water. But the agreement of this "model" (4.4 discrete O-O interactions surrounded by a uniform distance distribution) with the radial distribution function of water is poor (Fig. 1). The short-distance part of the first peak in the $D(r)$ curves is reproduced reasonably well, but the long-distance part is not. Since, for tetrahedral coordination, interactions with second neighbors are centered around 4.5 angstroms (where a broad maximum is observed in Fig. 1), the conclusion cannot be avoided that the first coordination sphere of a water molecule is complex, as stated in point D (*11, 12*).

At the time that data for the radial distribution curves of Fig. 1 were measured, small-angle scattering data for water were not available. For these curves the intensity functions were extrapolated to zero values at zero scattering angle (*8, 10*), a procedure which idealizes the radial distribution functions to those for constant bulk density at large values of r. Hence, the curves of Fig. 1 should not be considered reliable at $r > \sim 10$ angstroms. Recently several sets of small-angle scattering data have been reported (*13–16*), of which those of Levelut and Guinier (*16*) are the most accurate and extensive. These authors find that the scattered x-ray intensity for water at room temperature in the angular region $0.19 \leqslant s \leqslant 0.44$ is constant, with value $I(0)/nF^2$ equal to 0.064 ± 0.002. These results have been confirmed at Oak Ridge National Laboratory (*17*) to values of $s \geqslant 0.015$. These new measurements have at least two consequences for the structure of water:

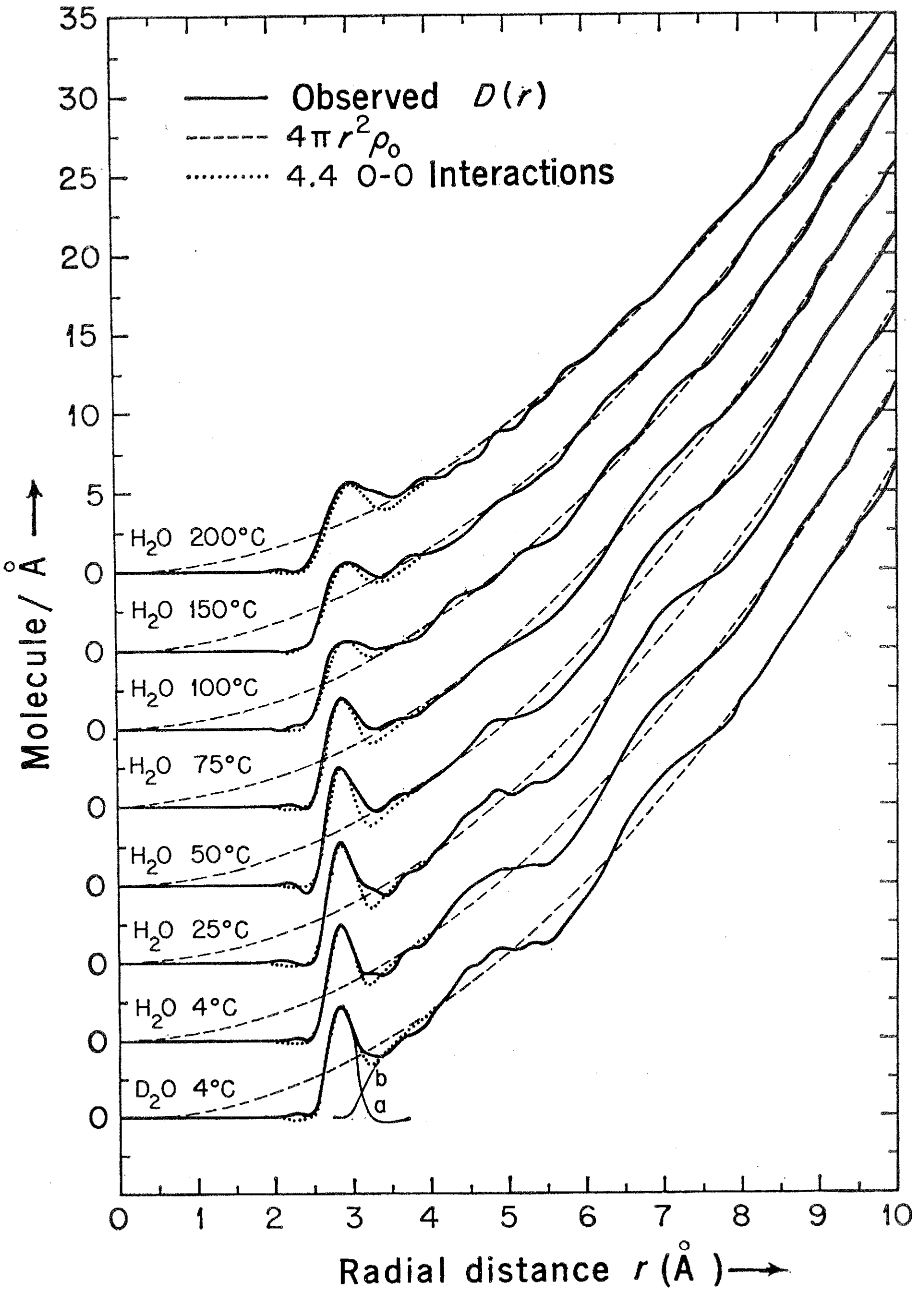

Fig. 1. X-ray radial distribution functions $D(r)$ for liquid water. The first major peak cannot be described by superposition of a single Gaussian distance distribution (*a*) and a continuum (*b*), because the resulting dotted curve does not fit the long-distance portion of the observed maximum.

E) The lack of interference maxima in the small-angle region precludes any significant variation in the pair density function not included in the curves of Fig. 1.

F) The value of the relative variance of the particle number from extrapolated zero scattering (Eq. 3a), 0.064, does not differ significantly from that predicted by Eq. 3b: $kT\rho_0\kappa = 0.0635$. The random fluctuations in density expected for a model consisting of a single phase and a single chemical species are thus sufficient to explain the observed small-angle scattering. The properties of any other sort of model would have to be adjusted to conform to this same value.

Any proposed model for liquid water that has features which contradict points A through F must be rejected on the basis of the experimental evidence.

Proposed Water Models

Considerable evidence (*2*) supports the idea that the hydrogen bonds in liquid water form an extensive three-dimensional network, the detailed features of which are short-lived. Such a structure may arise in a variety of ways from units of nearly tetrahedral symmetry. There are nine known modifications of ice (*18*), each stable over certain temperature and pressure ranges. There are numerous hydrate crystals in which the water molecules might be described as clathrate "ices" (*19*). In all the known ice modifications and most clathrate hydrates, each water molecule is hydrogen-bonded to four others. The small energy changes for the high-pressure ice transitions indicate that the four bonds with nearest neighbors are not critically sensitive to angular distortions as large as 30° from the tetrahedral angle. The question of whether hydrogen bonds are "broken" when ordinary ice melts, or are merely stretched and bent (as in the high-pressure ice transitions), is the subject of controversy.

"Continuum" models describe water as a hydrogen-bonded network with a continuous distribution of bond energies and geometries. Observations of the vibrational spectrum of liquid water, which is highly sensitive to the strength and extent of hydrogen bonding, have been interpreted both in support of (*20*) and against (*21*) this view. A description of this kind does not easily lead to a partition function and thus to a prediction of thermodynamic properties.

In "mixture" models it is assumed that an equilibrium exists between discrete molecular groupings with different numbers of hydrogen bonds per molecule. This equilibrium is temperature- and pressure-dependent, and formal descriptions in these terms account for many of the thermodynamic properties of water and aqueous solutions (*22–25*).

Results of diffraction studies may be cited in support of both views. Stretching of hydrogen bonds from 2.76 angstroms in the solid to an average of 2.82 angstroms in the liquid near the melting point accounts for most of the heat (1.4 kilocalories) necessary to melt one mole of ice. The fraction of "broken" bonds, if any, should therefore be small. On the other hand, the first peak of the radial distribution function of liquid water cannot be reproduced by a single Gaussian distance distribution; consequently, there are almost certainly at any instant at least two types of water molecules in the liquid, each with different near-neighbor configurations. However, the implication with regard to hydrogen bonding depends largely on the definition of a "broken" bond.

Distorted bond model. Pople (*26*) has made the only attempt to derive the radial distribution function for a "continuum" model. He assumed that after fusion the four hydrogen bonds from any one water molecule are able to bend independently, whereas in the solid phases they can only bend in such a way that the lattice order is maintained. A simple cosine expression was assumed for the energy of a bent bond and was used to obtain the probability distribution function of bond angles. The moments of this distribution function were then used to synthesize a radial distribution function composed of Gaussian-like components, which was compared to the observed functions of Morgan and Warren (*12*).

Pople's treatment describes the distribution of first (*B*), second (*C*), and third (*D*) neighbors around an arbitrary origin molecule (*A*), based on the assumption that the distribution of neighbors about any atom is independent of the distribution about the neighboring atoms. However, such as assumption must be incorrect because it is inconsistent with the necessary condition of geometric compatibility. The implications of this lack of independence are very difficult to analyze, and Pople makes no attempt to do so. It seems to us, however, that they are potentially so serious as to invalidate his conclusions. Even apart from these inconsistencies, it seems clear that a model based only on the hypothesis of independent bending of hydrogen bonds is not sufficiently detailed to permit the calculation of radial distribution functions having the distinct features of those observed for low-temperature water. In particular, the complexity of the first shell discussed earlier is not reproduced.

Flickering cluster models. Frank and co-workers (*22, 23*) and Nemethy and Scheraga (*24, 25*) have visualized liquid water as consisting of two or more "states" in equilibrium. Typically, one state consists of more or less ordered hydrogen-bonded molecules—the "bulky phase" or cluster—and another of nonhydrogen-bonded, disordered molecules—the "dense phase." The cluster is visualized as short-lived (10^{-10} to 10^{-11} second) and is continually exchanging molecules with the adjacent unstructured phase. The detailed arrangement of molecules within a cluster is never specified but it is usually visualized as possibly ice-like, and in any case as consisting of tetrahedrally coordinated molecules.

We have discussed the detail needed in order to test the validity of a cluster model with x-ray diffraction data, namely conditions 6 through 9. Although condition 7 is predicted from thermodynamic considerations, condition 6 is usually left unspecified since it is not needed in thermodynamic discussions; in its absence no detailed test with x-ray diffraction data can be made. There is, however, one proposed cluster model (*24, 25*) in which the specifications of conditions 6 through 8 have been given for near-neighbor interactions, and agreement with x-ray data is claimed.

Nemethy and Scheraga explain the first peak of the radial distribution function of water as due to interactions between two different near-neighbors, namely, interactions between hydrogen-bonded first neighbors (*A*) and interactions between first neighbors of unbonded molecules and of molecules located on the cluster surface (*B*). The numbers (*25*, Table V) indicate 2.05 interactions of type *A* per average water molecule and 3.89 interactions of type *B*, for water at 4°C. The type-*A* distance was taken to be 2.76 angstroms, as in ice, and that for type *B* was empirically fitted to the observed radial distribution function at 3.1 to 3.2 angstroms. Using these numbers, we found it impossible to reproduce the first peak in the radial distribution function of water at 4°C, since the predicted area of this peak is much too large. At higher temperatures the discrepancy appears to be even more serious.

Although the evaluation of cluster models against large-angle diffraction data is, at best, inconclusive, there are definite implications with regard to small-angle x-ray scattering. It has already been stated that experimental measurements are in close agreement with those predicted for random equilibrium fluctuations in density for individual water molecules. Thus the cooperative effect which is basic to the flickering cluster concept would appear to be absent. Only if the model should provide an extremely small density difference between the constituent "phases" could this conclusion be avoided. In particular, the difference of 20 percent assumed by Nemethy and Scheraga is clearly too great. Any ice-like cluster structure would also seem to have a larger density deviation than can be tolerated.

Gas hydrate model. In a large class of compounds known as clathrate hydrates (*19*), water molecules form hydrogen-bonded network structures in which, as in ice, the molecules are tetrahedrally or nearly tetrahedrally coordinated. These networks are less compact than that in ice, and they contain large polyhedral cavities which can accommodate guest molecules. Pauling (*27*) suggested that water has a short-range structure similar to that of some gas and salt hydrates ("water hydrate"). Figure 2 shows the hydrate structure of chlorine (*28*) which Pauling proposed as a possible starting point toward a model for liquid water. This model is sufficiently well defined to permit the calculation of radial distribution functions, and we have investigated it in detail.

The Pauling model (Fig. 2) assumes that all 46 network (*N*) and eight cavity positions of the cubic unit cell are occupied by water molecules. The cavity molecules are located at the centers of two dodecahedra (*D*) and the six tetrakaidecahedra (*T*). In order to be consistent with the density of water at 25°C (0.033 molecule/cubic angstrom), the Pauling model predicts, per water molecule, 3.41 O-O interactions of type *N-N* at a distance of 2.73 angstroms. In addition, there would be 0.59 interaction of type *N-D* at 3.72 angstroms and 0.89 interaction of type *N-T* at 3.89 angstroms. The x-ray radial distribution function of water at 25°C shows approximately 4.4 interactions at 2.85 angstroms, and thus the Pauling model as originally described is not compatible with the x-ray data.

The following modifications of the Pauling model, which retain the symmetry of the cubic gas hydrates, *Pm*3*n*, were considered:

1) Expansion of the clathrate network, accompanied by random occupancy of the cavities by more than one water molecule, again constrained to the experimental density (one independent distance variable).

2) Distribution of the scattering density of the cavity molecules over the surface of a sphere of appropriate dimension, so that the distance to the nearest network oxygen atom is a van der Waals distance. This corresponds to random location within the cavities but maintenance of an appropriate van der Waals contact with the nearest network molecules.

3) Localization of the cavity molecules at random at those points which are one van der Waals distance from the atoms of a polyhedral face (one independent distance variable).

4) Deformation of the clathrate cages and variation of the relative orientation of the dodecahedra (three independent distance variables).

Modification 4, which was tried first, yields four different near-neighbor distances of type *N-N*, which are both larger and smaller than the single Pauling distance of 2.73 angstroms. It did not improve the agreement with the diffraction data. We found it necessary to expand the clathrate network sufficiently so that the near-neighbor distances could assume values of at least 2.8 angstroms.

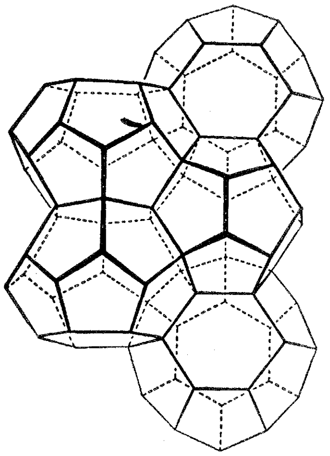

Fig. 2. Gas hydrate model. Only four of the twelve tetrakaidecahedra around a central dodecahedron are shown; oxygen atoms on the corners of the polyhedra form host network around large cavities which accommodate guest species.

Consideration of modification 1 indicates that the maximum network expansion consistent with the density of water at 25°C leads to an *N-N* distance of 2.86 angstroms and two water molecules in each cavity. Let us define a cavity radius as the distance from its center to the nearest network position. For an *N-N* distance of 2.86 angstroms, the radius of a dodecahedron is 3.90 angstroms. Placing the two *D* molecules equidistant from each other as well as from the nearest *N* molecules results in an O-O distance of 2.6 angstroms for these *D-D* and *D-N* interactions. We found it impossible to reconcile this short distance (even when modification 4 is applied) with the observed radial distribution function of water.

The largest near-neighbor distance of type *N-N*, achieved by placing one molecule in each dodecahedron and two in each tetrakaidecahedron, is 2.83 angstroms, and the radius of a tetrakaidecahedron is then 4.07 angstroms. This results in *T-T* and *T-N* distances of approximately 2.71 angstroms. Again we found it impossible to reproduce the first peak of the radial distribution function of water with this distance spectrum.

The common structural unit of all known gas hydrates is the pentagonal dodecahedron. The cubic configuration investigated by us is one of the simplest three-dimensional structures involving these building blocks. It seems unlikely that models based on the more complex gas hydrates would be more successful in explaining the radial distribution function of liquid water, since these solids differ from the structure discussed mainly in the way in which the dodecahedra are arranged in a three-dimensional repeating lattice, that is, in the relatively long-range order. The difficulties that were encountered with the cubic gas hydrate model are associated with nearest-neighbor distances.

In summary, the large dodecahedral and tetrakaidecahedral cavities with near-neighbor network distances in agreement with observation (2.85 angstroms) lead to unacceptably low densities. Multiple cavity occupancy to correct the density leads to unacceptably short distances involving cavity molecules. Thus the large cavities appear to be the critically unsatisfactory feature of the model.

Ice models. Even before the structure of solid water was known, Roentgen (*29*) suggested that ice-like domains were present in the liquid. Ordinary ice (ice-I*h*) is now known (*30, 31*) to have a structure similar to that of the hexagonal form of silicon dioxide, tridymite. Each oxygen atom (Fig. 3) is tetrahedrally surrounded by other neighboring oxygen atoms, forming layers of puckered six-membered rings. Two adjacent layers, related by mirror symmetry, form dodecahedral cavities with a radius of 2.95 angstroms. These cavities, though significantly smaller than those found in the clathrate hydrate structures (Fig. 2), are large enough to accommodate a water molecule.

Bernal and Fowler (*32*) interpreted early diffraction data on liquid water in terms of a mixture of tridymite- and quartz-like domains. The classical x-ray work of Morgan and Warren (*12*) led to the eventual abandonment of this widely discussed model.

Davis and Litowitz (*33*) have also proposed that water might be a mixture of two kinds of domains: an open type with an arrangement of molecules as in ice-I, and a more densely packed one with a structure similar to that of ice-II. However, Davis and Litowitz gave no specification as to how these two structures are arranged with respect to each other, and thus their model lacks the complete definition (conditions 8 and 9) necessary for comparison with diffraction data. Davis and Litowitz have made a calculation of the shape of the first peak for the radial distribution function of their model, but, since they consider only interactions between water molecules within each of the two structures, the claimed agreement with observation would be meaningful only if each of the structural domains is so large that interactions involving boundary molecules can be neglected. We do not consider it likely that the existence of such large domains was intended by the authors, but, if assumed, it would surely be in conflict with data from small-angle x-ray scattering.

In a similar manner, Kamb (*18*) suggested that liquid water might be a mixture of three types of clusters having structures similar to ice-I, ice-II, and ice-III. He showed that linear combinations of radial distribution functions calculated for the three ice structures reproduce the curves of Morgan and Warren (*12*) qualitatively. As in the case of the Davis-Litowitz model, the existence of large clusters is implied by a treatment of this kind. Such large clusters of configurations like ice-I, ice-II, and ice-III would give rise to much more small-angle scattering than that observed by Levelut and Guinier (*16*) and are thus ruled out.

Ice-I model. Detailed models of liquid water, based on a modified ice-I structure (Fig. 3), have been proposed by Samoilov (*34*) and by Forslind (*35*). These models have achieved considerable success in accounting for the properties of water with but a few assumptions (*36*). The liquid phase is regarded as an extensive hydrogen-bonded network, the details of which are short-lived. Over short distances from any origin molecule, this network is closely related to a slightly expanded ice-I lattice. The average structure of this network is very open, with spaces between the groups of molecules in tetrahedral coordination sufficiently large to accommodate additional water molecules (Fig. 3).

Danford and Levy (*37*) have shown that intensity and radial distribution functions calculated for the ice-I model agree with the x-ray data for water at room temperature. Subsequent studies at Oak Ridge National Laboratory (*8*) have demonstrated that the same model can reproduce the radial distribution functions of Fig. 1 at all temperatures, and that the temperature dependence of the model parameters is related to the thermodynamic properties of water in a physically reasonable way.

The complexity of the first coordination shell is explained by the model (*8*) in terms of the distinctly different *average* environments of network and cavity molecules. In terms of average configuration, the model would thus be classified as a "mixture" model, with the network molecules forming nearly four hydrogen bonds each and the cavity molecules interacting with the network by less specific but by no means negligible forces. However, both of these "species" exist in environments which are distorted from the average, and these distortions are implied by sizable root-mean-square variations in interatomic distance. On a scale of many molecules, the ice-I model is "one phase" and is homogeneous. There is no conflict with small-angle scattering measurements, since the superposition of interatomic distance distributions approaches a uniform distribution of distances at distances beyond about 8 angstroms.

Conclusions

Most of the proposed models for liquid water cannot be tested against diffraction data because they are not sufficiently defined at the molecular level. Of the structures which have a sufficiently detailed basis to permit calculation of radial distribution functions, only the ice-I model has been shown to give agreement with data from both large- and small-angle x-ray scattering. In the few other cases in which radial distribution functions have been calculated, the apparent agreement with experimental curves for liquid water is meaningless, since the computed curves do not correctly describe radial distribution in the respective models.

Any realistic model of liquid water must accommodate (or at least avoid conflict with) the significant features which can be deduced directly from the available diffraction data. In particular, the idea that water molecules form clusters sufficiently different in size, shape, density, and structure from other such aggregates, or from "unstructured" water, to warrant the dis-

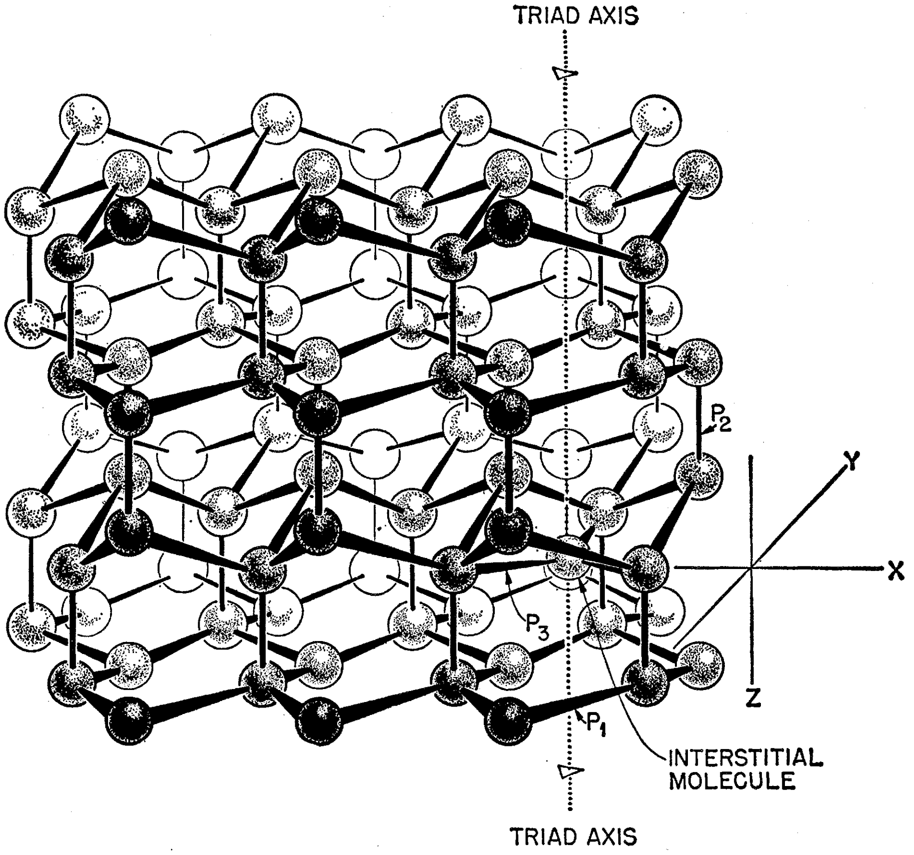

Fig. 3. Ice-I model. Oxygen atoms form six-membered puckered rings around cavities, which are not occupied in the solid (hydrogen atoms not shown). P_1, P_2, and P_3 are independent near-neighbor distances of the model, which assumes only short-range order of the type shown. The environment of a given water molecule in the liquid is distorted from the average.

tinction seems to be extremely difficult to reconcile with data from small-angle x-ray scattering.

If possible, proposed water models should specify the detail needed for comparison with diffraction data and should do this in a self-consistent way. If this is not done, the diffraction data cannot be invoked as evidence for or against the correctness of the model. Furthermore, any model that claims agreement with the observed diffraction pattern of liquid water should reproduce not only the first peak but all other significant features of the radial distribution function as well. These conditions provide a powerful criterion for the tenability of proposed water models.

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Mechanism of Lysozyme Action

Lysozyme is the first enzyme for which the relation between structure and function has become clear.

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For years, one of the most intriguing problems in biochemistry has been the mechanism of enzyme action. At the simplest level, without considering the problems of the regulation or coupling of enzyme systems, the aim has been to understand how enzymes achieve their great substrate specificity and to identify the detailed molecular mechanism of a given enzyme-catalyzed reaction. The reactions catalyzed by a number of enzymes, in particular proteolytic enzymes, have been investigated in great detail in an attempt to answer the above questions, and some of these enzymes are now reasonably well understood. It is an ironic testimony to the power of the x-ray crystallographic method, however, that lysozyme, for which there was no substrate of known structure only 12 years ago, and which for a long period of time was a "neglected" enzyme, is now one of the few clearly understood enzymes.

Lysozyme has had a rather peculiar history. In 1922 Alexander Fleming discovered a substance in his own nasal mucus capable of dissolving, or lysing, certain bacteria. The substance, which turned out to be an enzyme, was named "lysozyme" and was found to be widely distributed in nature (*1*). Fleming was quite enthusiastic about the possible therapeutic use of lysozyme—he had found it, in fact, because he believed that some organisms must produce antibacterial substances—but it soon developed that lysozyme was of little clinical value. Heartened perhaps by his initial partial success, Fleming went on to discover penicillin, the first true antibiotic, and interest in lysozyme fell into a relative decline.

Lysozyme was not completely forgotten though. The enzyme from hen egg white is easily isolated and purified, stable, and of rather low molecular weight (14,500), and as such it has become one of the most thoroughly investigated proteins (*2*). In 1963 Jollès and Canfield independently elucidated the complete primary structure

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