reflected from the parachute, which is made of partially metallized silk or Mylar, could also contribute, but our tests on the silk material indicate a very low reflectivity in the ultraviolet. Other potential problems, such as optical absorption in the expulsion charge gas cloud, should not lead to consistent results. Therefore, we have found it increasingly difficult to account for the anomalous results in terms of an instrumental problem.

If a real atmospheric effect is present, certain optical characteristics can be specified. They are (i) maximum extinction between 2950 and 3000 Å, (ii) steep gradient between 3000 and 3100 Å, (iii) decreasing extinction below 2900 Å, and (iv) total attenuation at 3010 Å near 25 percent (*10*) for the Wallops Island flight. The bandwidth would suggest an absorber rather than a scatterer.

The absorption spectra of many of the atmospheric constituents have been measured. None of these spectra appear to have the required characteristics. Of the constituents with unknown absorption spectra, most can be ruled out on the basis that a realistic cross section would require a minimum concentration of $\sim 10^9$ molecules per cubic centimeter to produce the observed intensity gradient. Two possibilities that remain are the metastable excited states of molecular oxygen, $b^1\Sigma_g{}^+$ and $a^1\Delta_g{}^+$ (*11*), which have been observed in the red and infrared airglow. A theoretical estimate (*12*) gives $O_2(^1\Sigma_g{}^+)$ concentrations barely above the acceptable limit. In the case of $O_2(^1\Delta_g)$, the distribution has been measured with a rocket flight from White Sands Missile Range (*13*). A sharply peaked distribution, with a maximum concentration of 3.6×10^{10} molecules per cubic centimeter at 49.5 km, was found. Use of this concentration with the 3010-Å intensity gradient at 52 km from the Wallops Island flight results in an absorption cross section of 9×10^{-18} cm^2, which is reasonable for an allowed transition. The total amount of $O_2(^1\Delta_g)$, based on this cross section, would be 4×10^{16} molecules per square centimeter, which is in substantial agreement with the White Sands total amount.

Although the ultraviolet spectra of these metastable states of oxygen apparently have not been determined, allowed transitions should exist from either state to a $^1\Pi_u$ state. The potential energy curve for this state is not known but is expected to be repulsive (*14*). The minimum photodissociation energy from the $^1\Delta_g$ state is 4.08 ev, corresponding to 3037 Å. The values for the $^1\Sigma_g{}^+$ state are 3.51 ev and 3531 Å, respectively. Until the character of the Π state is known or the absorption spectra are measured, it may be concluded only that one of the two transitions could have the correct energy and bandwidth for the anomalous absorption.

An explanation of the observed effects is of considerable importance to our work. At this time absorption by a metastable state of oxygen appears to provide a solution. The presence of such an absorber at 50 km will also affect other measuring techniques. A satellite method, using backscattered ultraviolet light from the atmosphere to determine the ozone distribution (*15*) requires precise information on all high-altitude absorbers in the spectral region from 2500 to 3400 Å. Neglect of a 3000-Å band absorber, above the ozone layer, would result in erroneously high ozone concentrations derived for the 30- to 40-km region. Standard measurements of total columnar ozone could be affected if this absorption were present beyond 3055 Å, the shortest wavelengths used in the Dobson spectrophotometer. In addition to effects on measurements, if the suggested excited oxygen absorption exists, an additional source of atomic oxygen would have to be considered in photochemical calculations of the composition of the stratosphere and mesosphere.

ARLIN J. KRUEGER*
Research Department,
Naval Weapons Center,
China Lake, California 93555

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11. A further possibility is absorption by vibrationally excited ground state O_2. A transition from the $v'' = 10$, 11, or 12 levels of the $X^3\Sigma_g{}^-$ ground state to the $v' = 0$ or 1 levels of the $B^3\Sigma_u{}^-$ state could have the correct energy. These electronic states produce the well-known Schuman-Runge bands in the case where $v'' = 0$. Population of the higher vibrational levels at 50 km could come from the reaction of O_3 with O.
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* Present address: Planetary Radiations Branch, Goddard Space Flight Center, Greenbelt, Maryland 20771.

9 April 1969; revised 12 August 1969

Structure of "Polywater"

Abstract. *A structure for "polywater" is proposed. It consists of hydrogen-bonded clusters of water molecules lying at the vertices of rhombic dodecahedra. This structure contains features which are less unattractive than those which are part of several earlier models.*

There have been recent speculations (*1–3*) concerning the structure of "polywater." Models proposed include: (i) tetrahedral clusters (*1*) of $(H_2O)_4$; (ii) square tetramers (*2*) of $(H_2O)_4$; (iii) planar hexagonal sheets (*3*); and (iv) (apparently) planar, highly branched polymer chains (*3*). Unsymmetrical $O{-}H \cdots O$ bonds were postulated in models (i) and (ii) (*1, 2*), with $O \cdots HOH \cdots O$ angles of 60° and 90°, respectively. Symmetrical $O \cdot\cdot H \cdot\cdot O$ bonds were postulated in models (iii) and (iv) with 120° angles formed by each pair of the three oxygen atoms in two adjacent hydrogen bonds; Lippincott *et al.* suggested (*3*) that these symmetrical bonds might result from resonance among various structures, such as, in part

$$\mathrm{O{-}H\cdots O} \leftrightarrow \mathrm{O\cdots H{-}O}$$

In none of these models are the angles between adjacent hydrogen bonds very close to the average value of about 109.5° observed in eight different forms of ice (*4*) and in numerous hydrates (*5*). The deviations in the two tetrameric models (*1*, *2*) are all so large as to suggest that these models are unacceptable. In the case of the two planar models (*3*), more satisfactory bond angles could doubtless be achieved were the planarity restriction to be relaxed (*6*), but the essential two-dimensional nature of these models renders them inherently improbable: a lamellar structure for this viscous material seems intrinsically less likely than one based on some sort of three-dimensional network, or polymeric units, or a combination of these two features.

There does not seem to be any evidence that any of the eight three-dimensional networks observed in the ices (*4*) could be assigned to "polywater." The hydrogen bonds in these structures are all of the unsymmetrical $\mathrm{O{-}H\cdots O}$ type, with oxygen-to-oxygen distances of 2.75 to 2.95 Å, whereas in "polywater" the infrared spectra are thought to indicate (*3*) hydrogen bonds of the symmetrical type $\mathrm{O\cdot\cdot H\cdot\cdot O}$. By analogy with the fluorine-to-fluorine distances in the symmetrical bifluoride ion, Lippincott *et al.* (*3*) assumed that the O-to-O distances in symmetrical $\mathrm{O\cdot\cdot H\cdot\cdot O}$ bonds would be 2.3 Å. This value is, however, too low: O-to-O distances of 2.40 to 2.49 Å in symmetrical $\mathrm{O\cdot\cdot H\cdot\cdot O}$ bonds have been observed, for example, in potassium hydrogen malonate (*7*), potassium hydrogen chloromaleate (*8*), chromous acid (*9*), and potassium hydrogen maleate (*10*). The increase in the O-to-O distance from 2.3 to 2.44 Å reduces the maximum density of 1.40 g/cm³ calculated by Lippincott *et al.* (*3*) for the planar structures to 1.24, a value considerably smaller than the maximum value of 1.4 reported by Deryagin and Churayev (*11*) for "polywater." This result provides additional evidence against the planar structures.

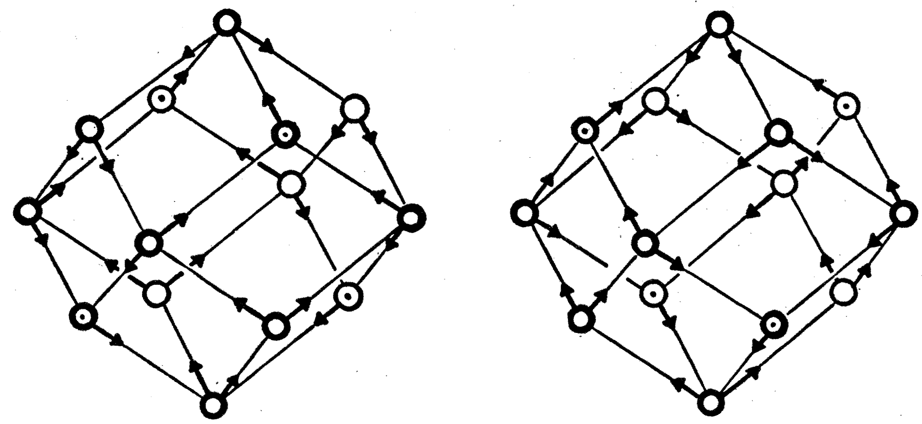

Fig. 1. Two canonical forms of a hydrogen-bonded rhombic dodecahedral cluster of $(H_2O)_{14}$. Circles represent oxygen atoms; arrows represent hydrogen atoms. The oxygen atoms which are bonded to the four hydrogen atoms not used in the formation of the cluster are indicated by the dots.

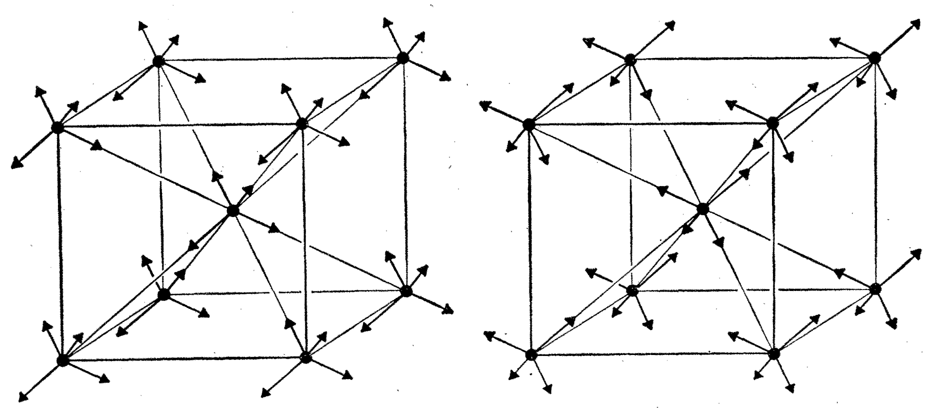

Fig. 2. Two canonical forms of part of a hydrogen-bonded supercluster. Filled circles represent rhombic dodecahedral clusters; arrows represent the "unused" hydrogen atoms of Fig. 1.

A cluster consisting of 14 water molecules located at the vertices of a rhombic dodecahedron does not suffer from the defects mentioned above. In this model all of the O-to-O angles can be 109° 28′. Two canonical representations of this structure are shown in Fig. 1; $\mathrm{O{-}H\cdots O}$ bonds are present along each of the 24 edges, and the four hydrogen atoms not used in the formation of the cluster are tetrahedrally directed. The clusters can thus be used to form larger polymeric units if they are positioned at the lattice points of a cubic body-centered lattice. These "superclusters" then may have two canonical structures, as depicted schematically in Fig. 2. An infinite supercluster with all $\mathrm{O\cdot\cdot H\cdot\cdot O}$ distances equal to 2.44 Å would have a density of 1.39 g/cm³; this value decreases as the size of the cluster increases, and it would also decrease if the hydrogen-bond distances between the clusters were to increase.

A not very dissimilar assemblage of polyhedral clusters occurs in chlorine hydrate, 46 $H_2O\cdot 6\ Cl_2$ (*12*). In this case the presence of Cl_2 induces the formation of a three-dimensional hydrogen-bonded network of water molecules lying at the vertices of nearly regular dodecahedra and tetrakaidecahedra, with the chlorine molecules in the interstices. The density of the water framework alone is 0.83 g/cm³. In the absence of Cl_2, the normal ice structure, without the large holes, prevails.

At present it does not appear to be possible to explain what could be responsible for the presence of rhombic dodecahedra in "polywater," if these do occur in that material, nor can the formation of symmetrical $\mathrm{O\cdot\cdot H\cdot\cdot O}$ bonds be accounted for, as these have been observed only in rather special circumstances (*7–10*). Nevertheless, the rhombic dodecahedral model contains features more attractive than those heretofore proposed.

JERRY DONOHUE

Department of Chemistry and
Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia 19104

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23 September 1969

Immune Response in vitro: Independence of "Activated" Lymphoid Cells

Abstract. *Antibody formation against sheep erythrocytes by mouse spleen cells in vitro requires interactions among antigen-treated macrophages and lymphoid cells in cell clusters for only a finite time. During this critical period of interaction, lymphoid cells become "activated" and thereafter can develop into antibody-producing cells independently of native antigen, macrophages, and cell clusters.*

The development of an immune response to sheep erythrocytes in vitro requires both macrophages and lymphoid cells. Macrophages, after exposure to antigen, stimulate lymphoid cells to develop into antibody-producing cells (*1*). The "stimulation" probably occurs through cell interactions in clusters. The continued integrity of these cell clusters is thought to be essential for the development of a maximum plaque-forming cell (PFC) response to sheep erythrocytes (*2*). We present data to support the concept that interactions among antigen-treated macrophages and lymphoid cells in cell clusters are essential only for a finite time during which the lymphoid cells become "activated." Thereafter, "activated" lymphoid cells destined to replicate and develop into antibody-producing cells are independent of macrophages, cell clusters, and native antigen.

The culture reagents, culture conditions, and cell population separation procedures used have been described (*1, 3*). Single cell suspensions from spleens of unimmunized, 6- to 8-month-old C57B1/6N mice were separated into macrophage and lymphoid cell populations (*4*). Macrophages (10^6 per dish) were incubated with 10^7 sheep erythrocytes (SRBC) for 60 minutes. After unbound erythrocytes were removed, the cultures were reconstituted with 10^7 lymphoid cells. Culture dishes were incubated for specified periods on a slowly rocking platform to facilitate both formation of cell clusters and cell interactions. Transfer of dishes to a stationary platform stopped formation of new cell clusters. Cell interactions were interrupted by transfer of the dishes to the stationary platform after dispersion of cell clusters by vigorous pipetting. After 4 days of incubation both clusters of cells and individual plaque-forming cells that release IgM antibodies were enumerated by a modification of the hemolytic plaque technique (*5*).

In this culture system, maximum PFC responses developed in cultures incubated on the rocking platform for 4 days (control maximum response, Table 1). After 6 hours of incubation cell clusters were visible with phase microscopy in these cultures and persisted throughout the 4 days. Cell-cluster plaques contained three to four PFC. In contrast, stimulated cultures incubated on the stationary platform for 4 days developed only minimum responses (control minimum response) similar to responses in unstimulated cultures. Rare, small cell clusters were visible, and cell-cluster plaques contained only about one PFC.

The conditions necessary for "activation" of lymphoid cells were determined by measuring effects on the PFC response, which result from manipulations on the cells in the cultures after specified periods of incubation. Transfer of the cultures from the rocking to the stationary platform after 48 hours of incubation with or without dispersion of cell clusters did not reduce the PFC response. Cultures transferred at 6 hours with or without dispersion of cell clusters developed only minimum responses, whereas cultures transferred at 24 hours developed responses that were one-third to one-half those of the control maximum responses. In cultures transferred without dispersion, cell clusters persisted and cell-cluster plaques had two to six PFC (Table 1). In contrast, in cultures transferred after dispersion, only rarely did cell clusters re-form, and cell-

Table 1. Plaque-forming cell responses of spleen cells to sheep erythrocytes under differing conditions of culture.

Culture conditions	PFC/10^6 recovered cells*	PFC per cell-cluster plaque†	Visible cell clusters	
			Number	Size
Control, 96 hours rocking				
+SRBC (control maximum response)	861	3.41	Many	Medium
−SRBC	66	—	Rare	Large
Control, 96 hours stationary				
+SRBC (control maximum response)	68	1.02	Rare	Small
−SRBC	40	—	Rare	Small
Hours rocking before transfer to stationary platform				
6	74	5.06	Rare	Small
24	380	5.92	Few	Large
48	890	2.33	Many	Medium
Hours rocking before dispersion of cell clusters and transfer to stationary platform‡				
6	60	1.70	Rare	Small
24	446	1.04	Rare	Small
48	1017	1.05	Rare	Small

* Values from a representative experiment. † Values approximating 1.0 indicate PFC are not in clusters. Values greater than 1.0 indicate PFC are in clusters. Each value represents the average of at least ten duplicate determinations from each type of culture. The minus size indicates less than ten determinations. ‡ Dispersion of cell clusters and returning cultures to the rocking platform at 6, 24, and 48 hours had no effect on the PFC response; the data (including average PFC per cell-cluster plaque and the number and size of visible cell clusters) were similar to the control maximum response and are not known.