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RAMAN AND INFRARED INVESTIGATIONS OF THE NATURE OF INTRACELLULA--ETC(U)
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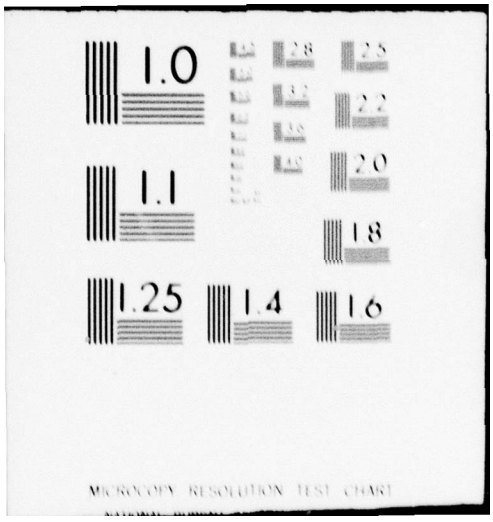
1.0
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OFFICE OF NAVAL RESEARCH

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Task No. NR 207-121

(9) TECHNICAL REPORT NO. 1

(6) "RAMAN AND INFRARED INVESTIGATIONS OF THE NATURE OF INTRACELLULAR WATER"

by

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ABSTRACT

Unpolarized infrared studies of the OH-stretching region were conducted for kangaroo tendon collagen fibers having water contents from maximum to minimum wetness. Spectral shapes were found to be much different than that from liquid H_2O. The OH-stretching region for all water contents shows the maximum absorption as a relatively sharp band near 3630 ± 20 cm.$^{-1}$ A broad maximum also occurs near 3490 ± 50 cm,$^{-1}$ followed by a weak broad shoulder near 3100 ± 100 cm.$^{-1}$ The OD-stretching region from dried collagen saturated with D_2O is somewhat weaker than the OH region. Nevertheless, a fairly sharp band occurs near 2660 ± 20 cm^{-1} and a broad band near 2350 ± 100 cm.$^{-1}$ The OH and OD features near 3630 ± 20 cm^{-1} and 2650 ± 20 cm^{-1} are assigned to strongly bent O—H···O and O—D···O units. The remaining broad OH and OD absorption maxima refer to strong more nearly linear hydrogen bonds. For H_2O all OH features seen at maximum wetness were detected after drying under vacuum with P_2O_5 for 136 hr. This and other observations indicate that water is strongly bonded to collagen in a way structurally unlike the hydrogen bonding present in pure bulk water.

1. Weight Loss and Infrared OH Spectra.

A. Weight Loss. The collagen fibers were immersed in water for 12 hr. The surface water was next rigorously removed with tissue and the fibers air dryed for 10 min. The fibers were then weighed. This weight corresponded to zero dessicating time. We next dessicated the fibers for 16 hr over P_2O_5 under vacuum and reweighed the fibers, and we repeated this procedure every 16 hr until 136 hr had elapsed. The dessication results are shown in Fig. 1.

From Fig. 1 it is evident that the initial weight loss was very fast. However, after 16 hr, or less, the rate of weight loss was nearly constant and equal to -0.858×10^{-4} g/hr, according to least square analysis. The rapid fall within 0 to 16 hr could, of course, result from the rapid loss of surface water or other loosely held water. However, some evidence to the contrary may arise from infrared spectra shown next. All infrared spectra were obtained at increments of 16 hr dessicating time. The first infrared spectra corresponded to zero time.

B. Infrared OH Spectra. Infrared spectra are shown in Fig. 2 for samples of collagen corresponding to (b) maximum wetness, zero dessicating time, and (a) minimum wetness, 136 hr of dessicating time. The spectra refer to per cent transmission versus vibrational frequency in cm^{-1} for the region from 2000 cm^{-1} to 4000 $cm.^{-1}$ The spectra were obtained by placing all of the collagen fibers in parallel contact with each other on a metal sample holder. The infrared Lambert absorption coefficient for OH stretching is extremely large, and usually necessitates very thin films, but the losses incurred because of the collagen absorption made the experiments simple, because only a relatively small amount of infrared radiation suffered OH absorption. Of course, scattering due to the collagen itself, and other effects, decreased the spectral quality somewhat.

Both the (a) and (b) spectra of Fig. 2 show visually similar shapes. Only the intensity of the (a) spectrum is lower because of the lower water content. Visual observation thus indicates that the main effect of drying is simply an intensity decrease due to loss of total water. However, computer analysis suggests that a component near 3490 ± 50 cm^{-1} may be concentration dependent, i.e., some loosely bound H_2O was lost in the early stages of the dessication. Both spectra of Fig. 2 indicate components near 3630 ± 20, 3490 ± 50, and 3100 ± 100 $cm,^{-1}$ which are the average values obtained in all infrared spectra of this work. Thus, from component positions alone, we see no great spectral differences between zero and 136 hr dessicating time.

For comparison with Fig. 2 the quantity $(\alpha\lambda_o/4\pi)$ for liquid water obtained by Crawford and Frech[1] is shown in Fig. 3(b). This quantity is roughly equivalent to an absorbance spectrum because λ_o does not vary much over the OH band, see the absorbance spectrum of Fig. 3(a) which was independently obtained by Walrafen.[1] Comparisons between Figs. 2 and 3 may be made despite the fact that one refers to a transmission spectrum and the other to an absorbance spectrum. These comparisons clearly indicate that the collagen OH spectrum contains many more contributions from oscillators in the 3400-3800 cm^{-1} region than liquid water. That is, the 3630 ± 20 cm^{-1} feature from water in collagen is much stronger than the corresponding liquid water feature, and there has also been a filling-in between about 3390 and 3630 $cm.^{-1}$

The comparisons between Figs. 2 and 3 clearly indicate that water in collagen must have a structure greatly different from that of liquid water.

2. Infrared Studies with D_2O in Collagen.

Samples of native collagen, that is, those not previously employed in the H_2O studies, were dessicated 2-3 days and then immersed in liquid D_2O for 2 days in a dry box. This procedure minimized H_2O-D_2O exchange, which had caused trouble in early work when samples saturated with D_2O were left in contact with room air. The native collagen samples were then rigorously dried inside the dry box with tissue, and transferred to the sample chamber of a Perkin-Elmer 180 infrared instrument which was purged with very dry nitrogen. An infrared transmittance spectrum is shown in Fig. 4.

The spectrum of Fig. 4 shows a relatively sharp feature near 2650 ± 20 cm,$^{-1}$ plus a broad band at 2300 ± 100 cm.$^{-1}$

When D_2O is added to native collagen, it is reasonable to expect that HDO will be formed by reaction of the D_2O with the H_2O already present in the collagen. Infrared absorbance spectra of HDO in liquid H_2O indicate OD components near 2610, 2510, and 2400 cm,$^{-1}$ see Fig. 5.[2] However, the 2610 cm^{-1} component is extremely weak and corresponds to a shoulder rather than to an absorption maximum--the absorption maximum occurs at about 2520 cm.$^{-1}$ Again, comparions between Figs. 4 and 5, indicates the spectrum of HDO in collagen to be greatly changed relative to HDO in liquid water. Much more intensity occurs at the higher frequencies, as in the case for H_2O in collagen, compared to liquid H_2O.

The ratio of isotope shifts that we have observed for the

sharp band from H_2O in collagen compared to the corresponding sharp band from HDO in collagen is 3630/2650 = 1.37. A value of 1.37 has been found experimentally to characterize OH/OD frequency ratios. (3) The frequency value for the strongest absorption of H_2O in collagen is 3490 ± 50 cm,$^{-1}$ and the corresponding value for HDO in collagen is 2350 ± 100 cm.$^{-1}$ Here the isotope ratio is 1.49, which is much too large. However, a broad absorption was also observed for H_2O in collagen at 3100 ± 100 cm.$^{-1}$ The HDO absorption at 2350 cm^{-1} may correspond to the unresolved sum of the bands at 3490 cm^{-1} and 3100 cm,$^{-1}$ as follows.

The ratio 3490/1.37 = 2547, and the ratio 3100/1.37 = 2263. Thus the value of 2350 for HDO in collagen lies between these two calculated values.

In any case the infrared spectrum of HDO in collagen is greatly different from that of HDO in liquid water, in complete agreement with our previous conclusion. Further, the 2350 cm^{-1} OD band from HDO in collagen is not far from that of HDO ice, (4) which indicates strong bonding between HDO and collagen.

3. Interpretation of Infrared Results.

As emphasized previously, the structure of bulk liquid water, and of water in collagen, are greatly different. Specifically, we find enhanced intensity at 3630 ± 20 cm^{-1} for H_2O in collagen, and at 2650 ± 20 cm^{-1} for HDO in collagen, compared to the bulk liquid phases. In addition we find bands for H_2O in collagen near 3490 ± 50 cm^{-1} and 3100 ± 100 cm^{-1} and for HDO in collagen near 2350 ± 100 $cm.^{-1}$

It is known from Raman and infrared studies of water,[1] aqueous solutions,[4] and from studies of OH in fused silica[5] that the OH and OD bands observed here refer to strongly bent hydrogen bonds. Similarly the remaining OH and OD bands from collagen refer to nearly linear hydrogen bonds. Linear hydrogen bonds indicate strong binding to collagen, as indicated particularly by the fact that the HDO value is close to that of HDO ice.[4] Beyond these gross observations the question arises as to how H_2O and HDO bind in collagen, that is, what specific structures explain the present infrared results.

Rich and Crick[6] describe a model of collagen in which one water molecule forms 2 hydrogen bonds between amide C=O groups. One amide group is in one polypeptide chain, and the other amide group is always that of glycine in a second polypeptide chain. The H_2O is thus situated such that the permanent electric dipole moment vector is perpendicular to the collagen fiber axis. Such an orientation is consistent with the infrared findings of Suzuki and Fraser,[7] as reinterpreted by us in the next section of this report.

The present findings are not inconsistent with the permanent

electric dipole moment vector being perpendicular to the fiber axis, but we need also to impose the additional conditions that one hydrogen bond is strongly bent, and the other one about linear. We might, of course, invoke a model in which more than one H_2O molecule exists between polypeptide chains. Still all of the dipole moments would have to be perpendicular to the fiber axis to be consistent with the infrared dichroic data of Suzuki and Fraser, and some hydrogen bonds would have to be strongly bent and others linear to be consistent with our data. Further, Rich and Crick make no mention of this further possibility.

If only one H_2O molecule is thus considered between polypeptide strands, our model would be that shown schematically in Fig. 6. In Fig. 6 each C=O group refers to a section of separate polypeptide chains. Also one hydrogen bond is linear, and the other one bent. The HOH angle, however, is close to 109° as in liquid water or ordinary ice.

4. Discussion of the Interpretation of Suzuki and Fraser.

Suzuki and Fraser[7] have reported infrared dichroic spectra for water in collagen. Their infrared data differ greatly from ours, for unknown reasons. (Probably the collagens are chemically different.) Aside from this difficulty, they assign a band at 3250 cm^{-1} to symmetric HOH stretching and a band at 3450 cm^{-1} to antisymmetric stretching. From numerous Raman and infrared studies of water,[1] it is virtually certain that the Suzuki and Fraser assignments are wrong. Antisymmetric stretching from liquid water occurs at least near 3550 cm^{-1} or much above,[8] and is certainly not assignable to the 3450 cm^{-1} frequency. Hence, the transition moments from both of Suzuki and Fraser's reported bands are parallel to the H_2O symmetry axis, contrary to their assignments. And both bands show dichroism perpendicular to the fiber axis. Suzuki and Fraser simply obtained redundant data without realizing it. Our reassignment means that the permanent electric dipole moment vector of H_2O in collagen is perpendicular to the fiber axis. This, of course, says nothing about where the line between the H_2O protons is located relative to the fiber axis, contrary to the interpretation of Suzuki and Fraser. Our only further suggestion relates to linear and bent hydrogen bonds formed with amide C=O groups as shown in the illustration, Fig. 6.

5. Future Studies.

In future work with H_2O and HDO in collagen, we expect to conduct polarized infrared studies of the infrared bands reported here, that is, we will conduct infrared studies of the 3630, 3490, and 3100 bands from H_2O and of the 2650 and 2350 cm^{-1} bands from HDO in collagen. We are also considering the possibility of obtaining laser-Raman spectra of thin sections of collagen. We expect to try to mount a large number of sections cut perpendicular to the fiber axis on a circular plate, holding them down with thin plastic film. This plate would then be spun in the laser beam, and the Raman scattering collected with a double monochromator. After this we plan to initiate polarized infrared studies of water in oriented skeletal muscle fibers.

6. References.

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2. G. E. Walrafen, J. Chem. Phys. 48, 244(1968).

3. G. E. Walrafen and L. A. Blatz, J. Chem. Phys. 56, 4216(1972).

4. G. E. Walrafen, J. Chem. Phys. 55, 768(1971).

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6. A. Rich and F. H. C. Crick, J. Mol. Biol. 3, 483(1961).

7. E. Suzuki and R. D. B. Fraser, in "Peptides, Polypeptides, and Proteins," ed. by E.R. Blout, F.A. Bovey, M. Goodman, and W. Lotan, John Wiley and Sons (1974), pgs. 449-458.

8. W. F. Murphy and H. J. Bernstein, J. Phys. Chem., 76, 1147(1972).

CAPTION

Figure 1. Dessication study of water in collagen. Zero time refers to collagen that has been soaked in water and then dried.

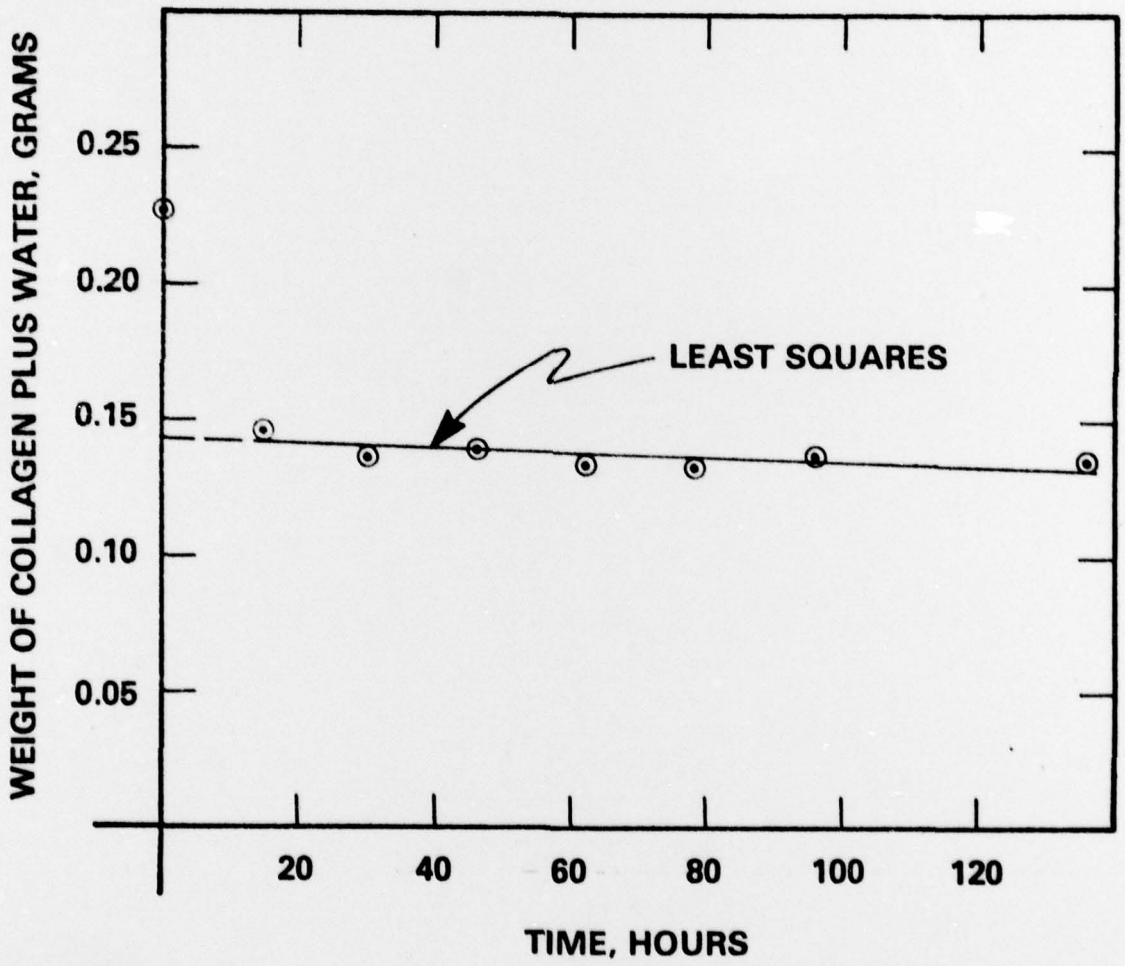
WEIGHT OF COLLAGEN PLUS WATER, GRAMS
0.25
0.20
0.15
0.10
0.05
LEAST SQUARES
20
40
60
80
100
120
TIME, HOURS

Figure 1

CAPTION

Figure 2. Infrared transmittance spectra of water in collagen. (A) Spectra of dessicated collagen with dessicating times indicated. (B) Spectrum corresponding to zero time, see Figure 1.

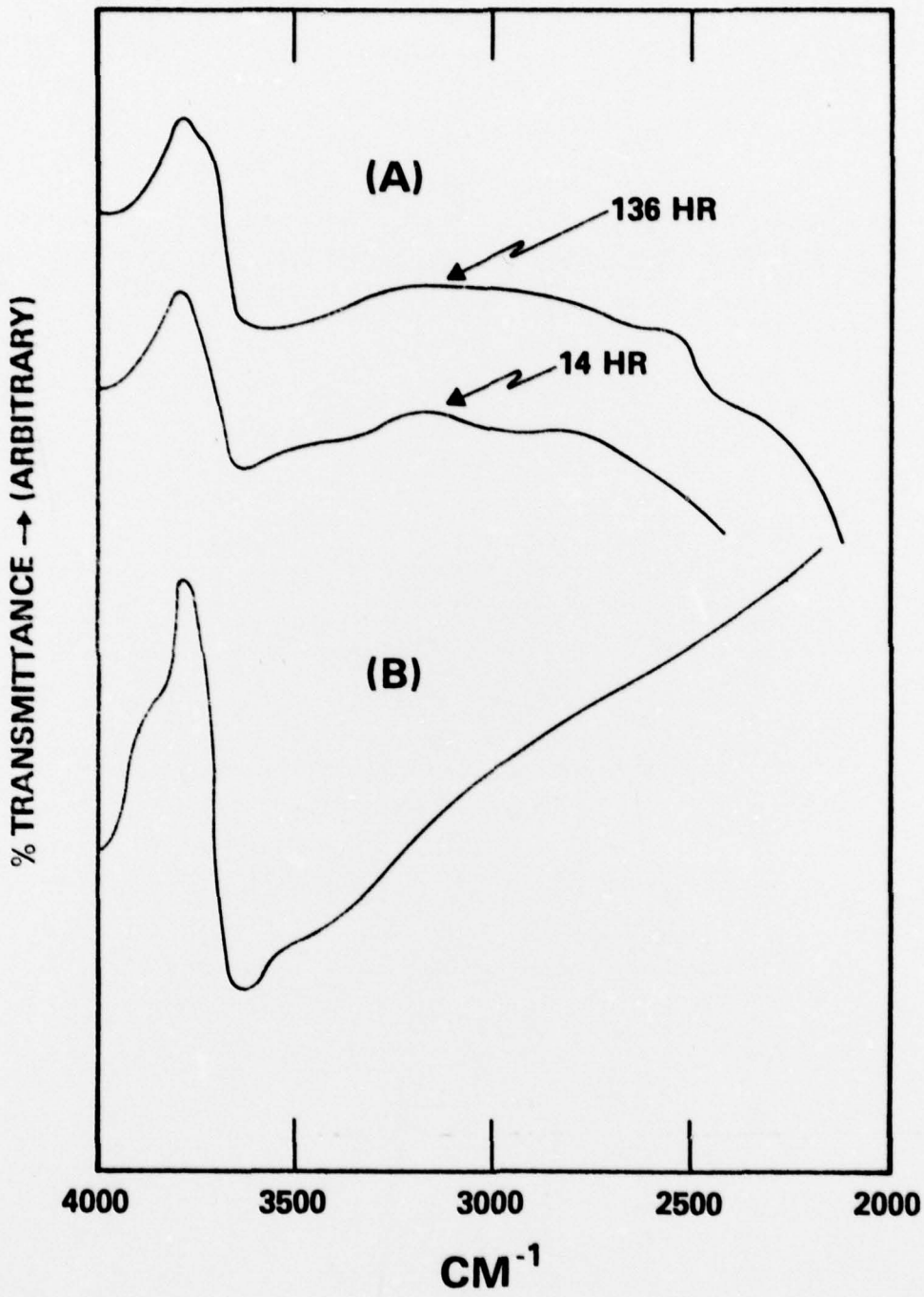
(A)
136 HR
14 HR
(B)
% TRANSMITTANCE → (ARBITRARY)
4000
3500
3000
2500
2000
CM-1

Figure 2

CAPTION

Figure 3. Infrared absorbance spectrum of liquid water, (a). Data obtained by Frech and Crawford,[1] (b). The (b) data are more accurate than the (a) data, but both sets of data compare favorably. Note the weakness of the absorption above 3500 cm^{-1}.

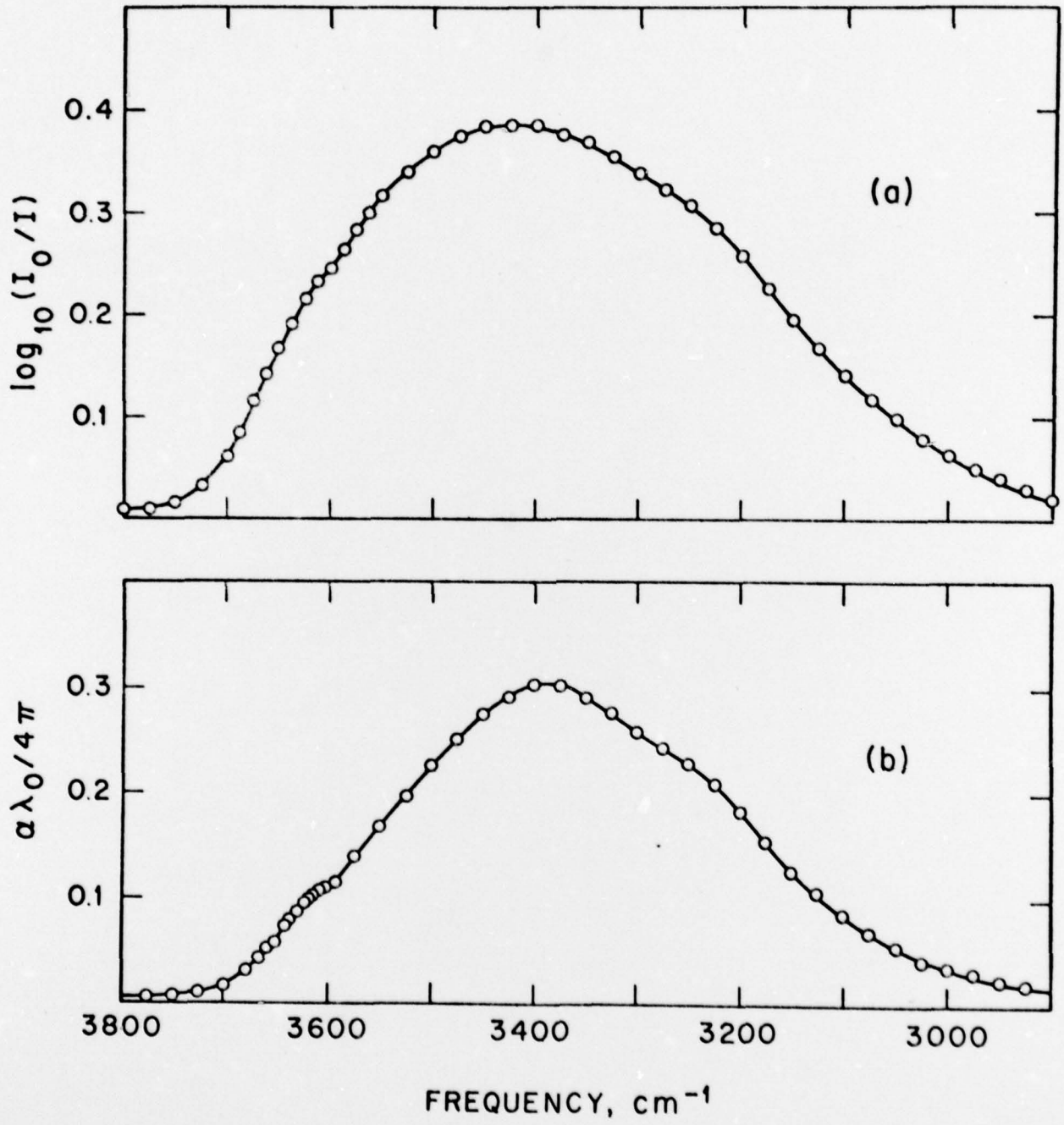
0.4
0.3
0.2
0.1
$\log_{10}(I_0/I)$
(a)
0.3
0.2
0.1
$\alpha\lambda_0/4\pi$
(b)
3800
3600
3400
3200
3000
FREQUENCY, cm^{-1}

Figure 3

CAPTION

Figure 4. Infrared transmittance spectrum of dry collagen to which D_2O has been added. The OD region indicated on the figure refers to HDO. The OH region contains contributions from both H_2O and HDO.

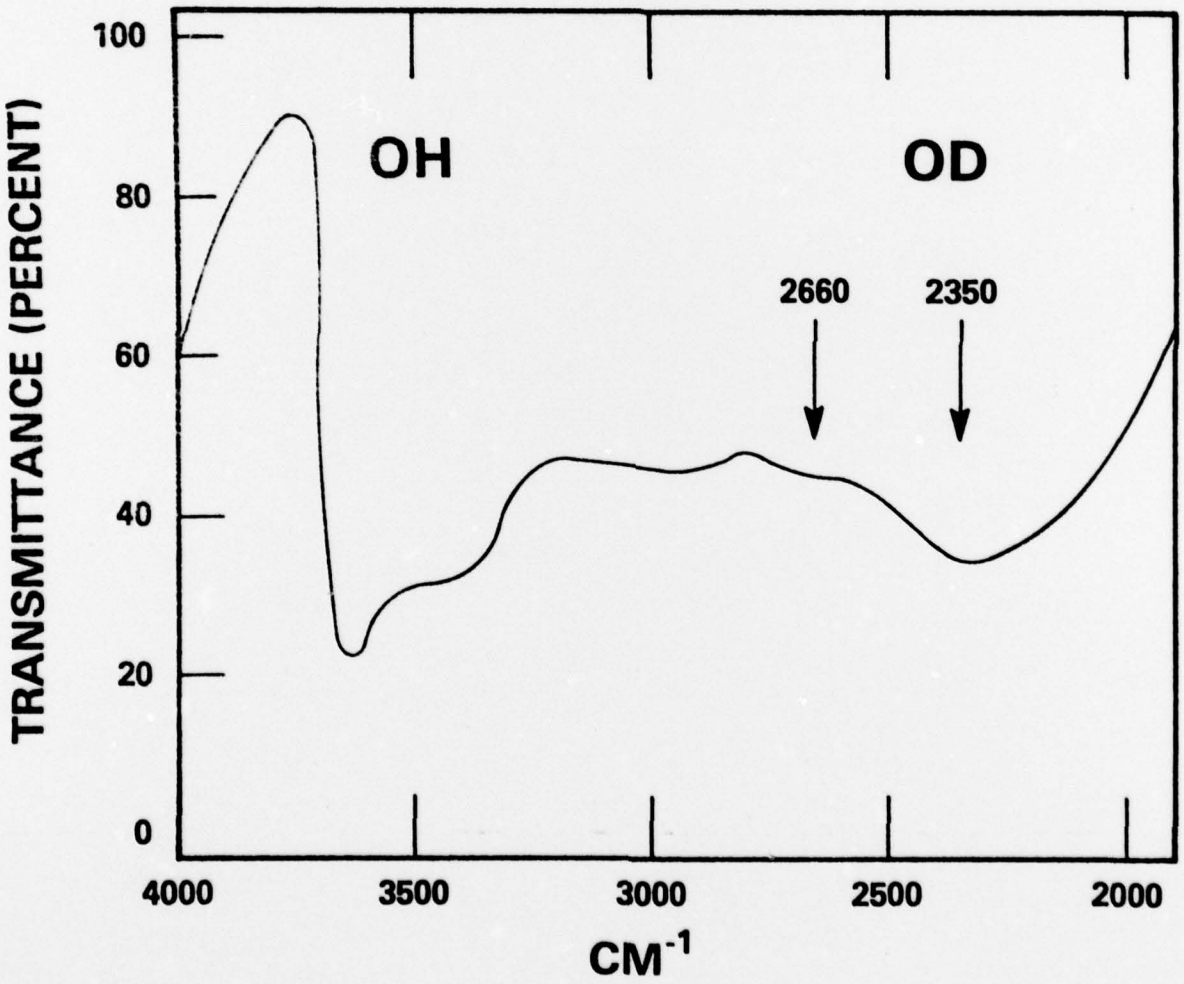
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
OH
OD
2660
2350
4000
3500
3000
2500
2000
CM-1

Figure 4

CAPTION

Figure 5. Infrared absorbance spectrum of the OD-stretching region of HDO in H_2O. The OD-stretching region region occurs between about 2100 to 2700 $cm.^{-1}$ The vertical lines under the main peak near about 2520 cm^{-1} refer to the centers of Gaussian components. The horizontal lines refer to the half-widths of the Gaussian components.

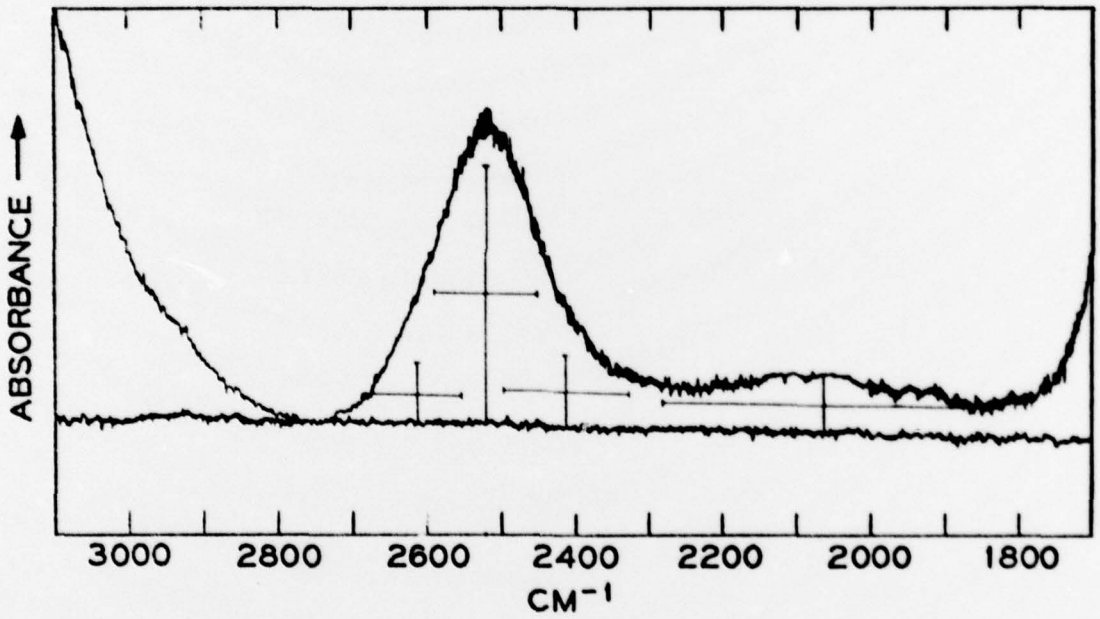

Figure 5

CAPTION

Figure 6. Schematic representation of the binding of a water molecule between two polypeptide strands of collagen. The dots refer to hydrogen bonds. The arrow refers to the direction of the permanent electric dipole moment of the water molecule. The dashed circles refer to the polypeptide strands, looking down the strands. Notice the linear and bent hydrogen bonds.

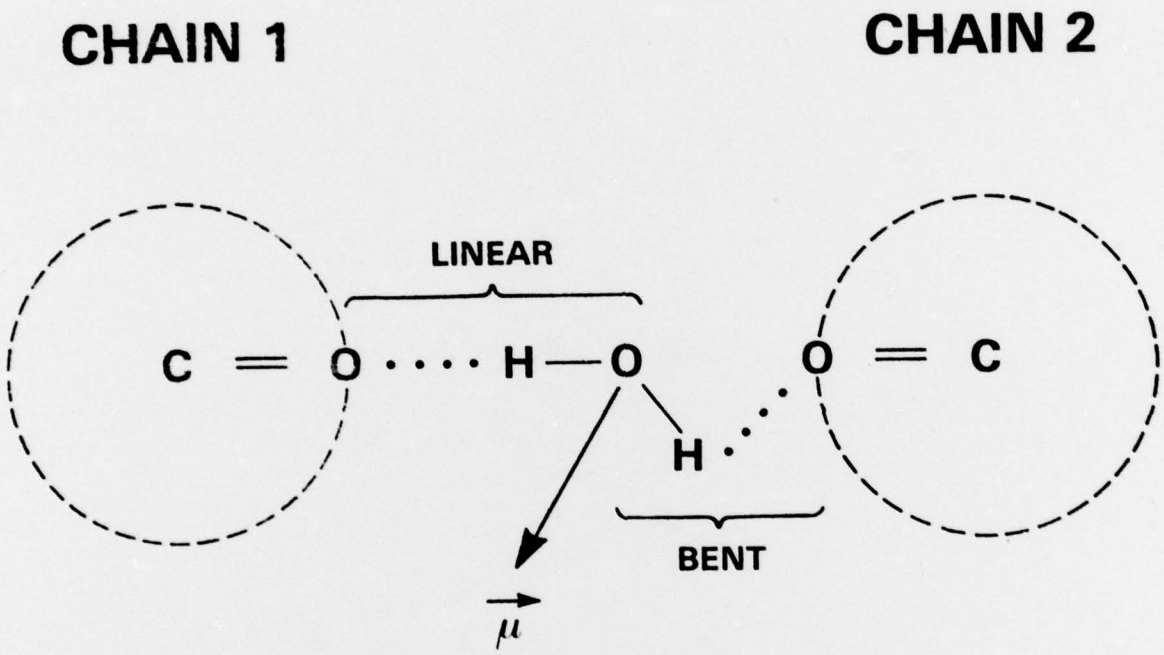

Figure 6

Budget, Second year.

Budget Category	ONR Funded Man Months	Prop. Amt. $
A. Salaries		
1. Post-doctoral Res. Associate	100% 12FTE	$15,000
B. Staff Benefits 20% Salaries		3,000
C. Total Salaries and Staff Benefits		18,000
D. Travel		500
E. Publication Costs		1,000
F. Laser Repair		3,000
G. Supplies		775
H. Indirect Cost		
a.) 82.8% of Salaries		12,420
I. TOTAL SECOND YEAR		$35,695

Note; The principal investigator, Dr. George E. Walrafen, will work on the project without charge, for the full grant period of 12 months.

"The salaries in this proposal are stipulated salaries as defined in Federal Management Circular (FMC)73-8, Section 7c."