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Modifications in the Near Infra-Red Absorption Spectra of Protein and of Light and Heavy Water Molecules When Water is Bound to Gelatin

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Comparisons are made between the near infra-red absorption spectra of protein molecules in oven-dried gelatin and gelatin saturated with light and heavy water vapor respectively. The intensity of the first overtone valence N−H band at 1.50μ is greatly diminished by the addition of H_2O molecules, and this band is almost completely destroyed when D_2O molecules are taken on. The combination deformation-valence N−H bands at 2.05μ and 2.18μ are only partially destroyed in either instance. These facts suggest that the dipole moment oscillation of the N−H group constituting a part of the inter-molecular hydrogen bridge, C=O···H−N, is reduced when this bridge is broken and water molecules themselves become bridged to the N−H group; that the transverse dipole moment change is less affected than the parallel change; and that D_2O is much more effective than H_2O.

The 1.72μ and 2.28μ C−H bands do not change appreciably in intensity. However, at least the former shifts toward higher frequencies when water is taken on, indicating that the C−H groups are in a more vapor-like environment and corroborating the usual assumption that water molecules are attached only to the polar groups of the gelatin molecules.

The destruction of some of the usual absorption on the short and the long wave sides of the 1.44μ ($\nu_\sigma+\nu_\pi$) and 1.93μ ($\nu_\sigma+\nu_\delta$)H_2O bands indicates the nonexistence, or a reduction in number, of unperturbed vapor-like molecules as well as of the more highly perturbed molecules involving three and four hydrogen bridges. The presence of a sharpened and enhanced 1.79μ ($\nu_\sigma+\nu_\delta+\nu_R$) band indicates hindered rotation of H_2O molecules in a field more homogeneous than that in liquid water but having the same average value. A new weak band appears at 1.35μ and is believed to be ($\nu_\sigma+\nu_\pi+\nu_R$). Somewhat similar results occur with D_2O, requiring for complete interpretation, however, frequency contributions from hindered translation ν_T.

DURING the preparation of a monograph by Professor O. L. Sponsler on the subject of a molecular basis for a conception of protoplasm, embodying among other results those obtained at this institution by means of x-ray diffraction analysis, the question has arisen whether the methods of infra-red absorption spectroscopy can be used to corroborate some of the conclusions arrived at by other types of experimentation and reasoning. In particular, can use be made of the effects of hydrogen, or proton, bridging[1] upon certain well-identified absorption bands of molecules? We believe that the results thus far obtained indicate that these effects can be used.

The important types of protoplasmic materials are proteins, carbohydrates, fats and water. The scope of our investigations includes at least the first two and their interactions with water. Some preliminary results have been obtained with the protein of silk fibers and with the carbohydrates, sucrose crystals[2] and ramie cellulose fibers, using polarized infra-red waves in the region 1–2.5μ. Results which we believe to be more conclusive than these have been obtained with the protein gelatin, using unpolarized light. It is these results which are presented in the present paper. A preliminary report of this study already has been made.[3] Because of the variety in number and the complexities in form of the amino acid residues attached to the backbone of these long protein molecules in gelatin, as compared with the simple glycine and alanine residues of silk, and because of the presence of proline linkages in the backbone, the system being dealt with is complex. In spite of its complexity, gelatin is a protein upon which many different types of physical and chemical studies have been made. As a consequence, certain fairly definite conclusions have been deduced which lend themselves to spectroscopic verification.

In spite of the complexity of the gelatin molecule, its absorption spectrum in the 1–2.5μ region is relatively simple. In this region one has to deal

[1] For a comprehensive discussion of hydrogen bridges in organic compounds see Huggins, J. Org. Chem. **1**, 407 (1936). For recent summaries of and pertinent references to papers dealing with the spectroscopic consequences of hydrogen bridging see Gordy, J. Am. Chem. Soc. **60**, 605 (1938); also Errera and Sack, Trans. Faraday Soc. **34**, 728 (1938).

[2] Ellis and Bath, J. Chem. Phys. **6**, 221 (1938).
[3] Ellis and Bath, J. Chem. Phys. **6**, 108 (1938).

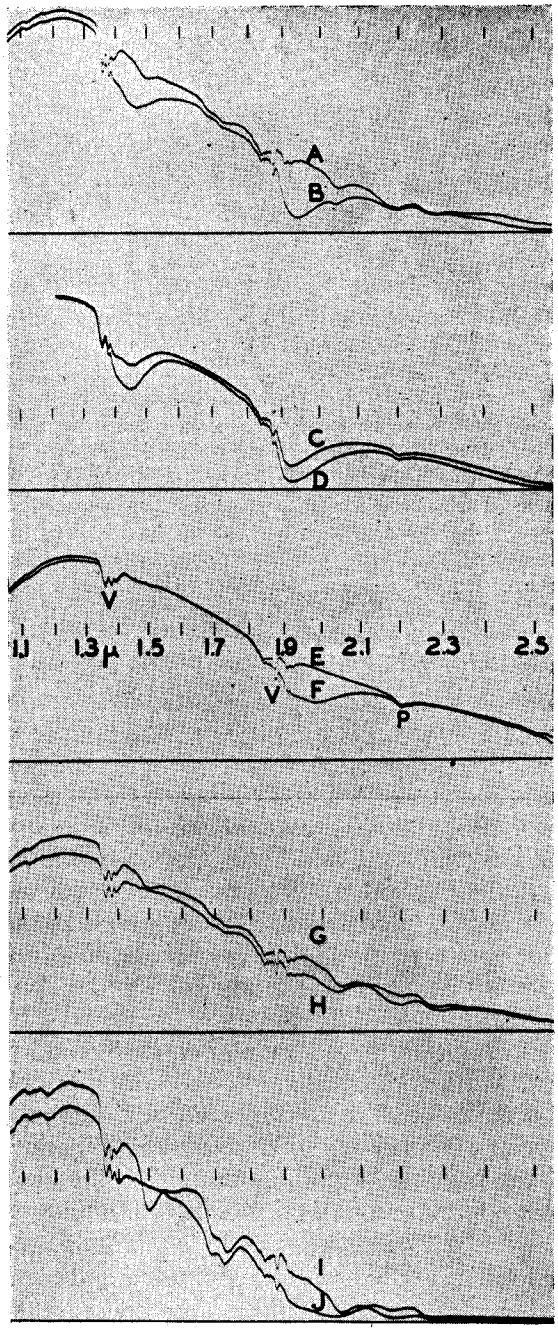

FIG. 1. A: 0.20 mm oven-dried gelatin. B: 0.28 mm gelatin+H_2O (weight of water 35.7 percent of total). C: 0.09 mm light water. D: 0.17 mm light water. E: Background distribution for F. F: 0.08 mm heavy water. G: 0.20 mm oven-dried gelatin. H: 0.30 mm gelatin+D_2O. I: 0.99 mm oven-dried gelatin. J: 1.49 mm gelatin+D_2O.

primarily, and perhaps entirely, with absorption bands associated with the overtone and combination vibrations of hydrogen atoms bonded to carbon, nitrogen and perhaps oxygen atoms. The only other type of oscillator which may have to be considered is the carbonyl group C=O. If the protein molecule is regarded as made up of approximately 300 amino acid molecules, then there exist on the backbone of the molecule this number each of C−H and C=O groups and about three-fourths of this number of N−H groups. The side chain residues contribute some thirty-one NH_2 groups, thirty-eight N−H, forty-nine O−H, eleven C=O groups and a large number of C−H pairs arising from CH, CH_2 and CH_3 groups.

In Fig. 1A is presented the absorption spectrum of a specimen composed of two sheets of gelatin, total thickness 0.20 mm, which had been oven-dried to constant weight at a temperature of 120°C. This spectrum was produced by the recording quartz spectrograph of the laboratory and is complicated by the presence of water vapor bands, *V*, of the atmosphere and by a quartz band, *P*, arising from the prisms. These vapor bands do not introduce appreciable difficulty when using such a spectrum with an appropriate background spectrum from which to obtain a percentage transmission curve, provided there is no change in the humidity of the room during the recording of the two spectra. Although there is no saving of time accomplished through the use of a recording instrument when it comes to reducing the records to a percentage transmission basis, nevertheless there is an obvious advantage in the use of such an instrument when the absorbing specimen is as optically imperfect as the typical sheet of gelatin. In the usual method of taking alternate galvanometer deflection readings with and without the specimen it becomes difficult to satisfactorily duplicate the alignment of the specimen in front of the slit.

Figure 1A shows five absorption bands of gelatin, none of them sharp, which we now interpret. The first overtone valence vibration band of NH and NH_2 occurs at 1.50μ. A band in this region is characteristic of primary and secondary amines[4] and its position in the spectrum is not particularly sensitive to the structure of the molecule. Similarly, the first overtone valence vibration band of CH, CH_2 and CH_3 groups oc-

[4] Ellis, J. Am. Chem. Soc. **49**, 347 (1927); **50**, 685 (1928).

curs at 1.72μ. At 2.05μ and 2.18μ are found bands arising from combinations of the fundamental valence and deformation vibrations of NH and NH_2; such bands also occur in the spectra of primary and secondary amines[3] and are structure-sensitive. In the spectrum of a molecule having an axis of symmetry coincident with the line of an N—H pair these two bands should coincide because of the degeneracy in the deformation vibration. A band resulting from a combination of the fundamental valence and deformation vibrations of C—H groups is found at 2.28μ. No bands characteristic of O—H groups appear, probably because there are few such groups. No apparent C=O band is found in this record; any C=O contribution falling within this region would arise from higher orders of overtones than are associated with the five bands just cited. Neither in this nor in the spectra of thicker oven-dried samples which we have studied do we find any evidence of residual water in the gelatin. This leads us to believe that the band at 3μ found by Buswell, Krebs and Rodebush[5] in the spectrum of a similarly prepared specimen and attributed by them to residual water in reality is produced by N—H valence vibrations.

When a gelatin sheet is allowed to stand in an atmosphere of ordinary water vapor it absorbs a maximum amount of water equal to about 35 percent of the total resulting weight. Such water is said to be bound to the gelatin. This process is accompanied by a 40 percent increase in thickness and a 6 percent increase in area. The amount of water taken on is the amount anticipated, provided every C=O, N—H, NH_2 and O—H group, unless restricted by space limitations, has attached to it the maximum number of water molecules possible through the mechanism of hydrogen bridging. It was anticipated, therefore, that if the spectrum of such a swollen sample were recorded and the absorption bands of the bound water plotted through a comparison of the records for dry and saturated gelatin specimens, differences should appear between these bands and those characteristic of an equivalent amount of ordinary water. Fig. 1B shows the record for such a specimen.

In Fig. 2A there is plotted against wave-lengths the percentage transmission of a 0.08 mm layer of ordinary distilled water at 22°C. This was obtained by dividing the ordinates in a 0.17 mm record, Fig. 1D, by those in a 0.09 mm record, Fig. 1C, and multiplying throughout by a factor obtained from the ratio of ordinates at some wave-length where there is no absorption. This procedure corrects for an inability to adjust the galvanometer deflections between two records and also corrects for reflection losses. In this spectrum are seen the well-known broad bands at 1.44μ ($\nu_\sigma+\nu_\pi$) and 1.93μ ($\nu_\sigma+\nu_\delta$) as well as the weaker band at 1.79μ ($\nu_\sigma+\nu_\delta+\nu_R$). Here ν_π and ν_σ are the frequencies of symmetrical and unsymmetrical valence vibrations, respectively, ν_δ and ν_R those of deformation vibration and hindered rotation respectively. The width of the slits is indicated by the width of the vertical line marked S on the graph.

In Fig. 2B is the percentage transmission curve for an amount of water equivalent to that in Fig. 2A, but bound to gelatin. It was obtained by a reduction of the data of Figs. 1A and 1B. Several significant differences between the spectra of Fig. 2A and 2B appear. Although the gelatin-bound water band at 1.44μ is slightly deepened, portions of both its short and long wave-length sides are missing. The sharpening on the short wave side was anticipated and is associated with the absence of free vapor-like molecules. It is

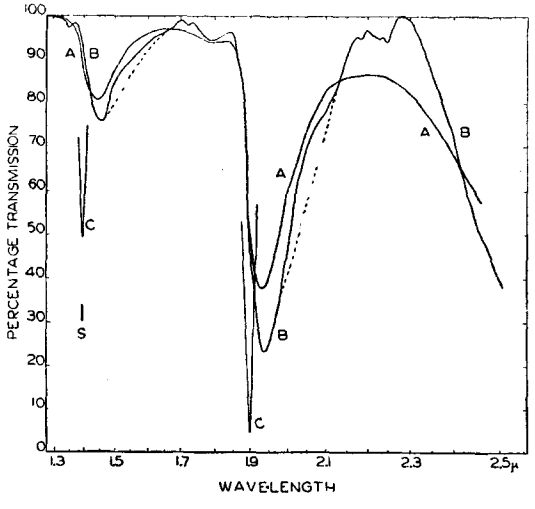

FIG. 2. A: 0.08 mm light water. B: 0.08 mm gelatin-bound light water. C: H_2O in CCl_4.

[5] Buswell, Krebs and Rodebush, J. Am. Chem. Soc. **59**, 2603 (1937).

believed[6] that there are some free unassociated molecules in liquid water. That the extreme short wave side of the band is the location for the contribution of free molecules is indicated by the sharp band plotted in Fig. 2C which represents the greatly enhanced central Q-branch[7] as it appears in the partially resolved vapor-like rotation-vibration band of water molecules in dilute CCl_4 solution.

In our interpretation of the alteration of the long wave portion of the 1.44μ band, we are guided by an analysis of the Raman bands of water by Cross, Burnham and Leighton.[8] They regard the water-vapor molecule so far as its valence vibrations are concerned, as made up of two coupled O−H oscillators, and the molecule of liquid water as such a molecule perturbed through hydrogen bridges by one, two, three or four other molecules. Their picture of the liquid water state is that of Bernal and Fowler.[9] Cross, Burnham and Leighton assume that the shift of frequency from the vapor position resulting from perturbation is greater when the oscillating molecule is the donor of the hydrogen in the bridge than when it is the acceptor. They also assume that the effects of perturbation are additive, the molecules which undergo the maximum fourfold perturbation contributing to the portion of the band furthest away from the vapor-like position. The absorption throughout the region 1.6–1.7μ in Fig. 2A is real and must be interpreted as the contribution to the 1.44μ band by molecules which are most highly perturbed, that is, perturbed by three and four hydrogen bridges. The fluctuating conditions of the liquid state can account for the continuity of absorption in such a band. The total width in frequency units of the 1.44μ ($\nu_\sigma+\nu_\pi$) band is considerably greater than the width of the Raman band (ν_π) or of the infrared band (ν_σ) in the 3μ region.[7] This is to be expected from the theory of perturbation. In fact, a comparison of the separation $\Delta\nu$ of perturbed and unperturbed O−H bands of alcohols in the fundamental[10] and first overtone[11] regions shows that the latter is about 13/5 as great as the former. This is the amount predicted by the quantum mechanics first order perturbation expression, $\Delta\nu \propto (2v^2+2v+1)$, in which v is the vibration quantum number.

The disappearance in Fig. 2B of some of the absorption in the 1.6–1.7μ region indicates that the gelatin-bound water molecules are in general not subjected to three and fourfold perturbations. This is consistent with the assumed model, which permits the absorbing water molecule to have no more than a twofold perturbation by N−H and C=O groups. The 1.44μ band in Fig. 2B seems to have two distinct parts, indicating two distinct types of perturbation. We shall show later that this interpretation is incorrect.

In the 1.93μ ($\nu_\sigma+\nu_\delta$) band of Fig. 2A–C occur effects similar to those in the 1.44μ band, and we offer similar explanations. The alteration of the long wave portion is even more noticeable than in the previous instance. The lesser alteration of the short wave portion, resulting in a smaller displacement of the whole band than in the 1.44μ band, is to be expected; for, the 1.93μ band should show some of the characteristics of both fundamentals, ν_σ and ν_δ, of which it is the combination. As pointed out by Cross, Burnham and Leighton,[8] and perhaps as shown even better by the hydration experiments of Ganz,[12] ν_δ behaves oppositely to ν_σ in regard to shifting when perturbed through hydrogen bridging, That is, ν_δ shifts to a higher frequency.

The behavior of the 1.79μ ($\nu_\sigma+\nu_\delta+\nu_R$), as displayed by Fig. 2A–B, is interesting. The existence of this band was first discovered by one of us,[13] was later challenged by Miss Kellner,[14] and finally was made a subject of special study by Collins.[15] It arises from simultaneous excitations of oscillation frequencies ν_σ and ν_δ and the hindered rotation frequency ν_R (510 cm^{-1}). It is interesting to notice that this band has the same location in liquid and gelatin-bound water, but is sharpened and intensified in the latter instance. These facts indicate that the potential field in which the absorbing molecule swings in hindered rotation in the gelatin medium is of the same magnitude but more homogeneous than in water.

[6] Kinsey and Ellis, Phys. Rev. **51**, 1074 (1937).
[7] Ellis and Kinsey, Phys. Rev. **54**, 599 (1938); also reference 6.
[8] Cross, Burnham and Leighton, J. Am. Chem. Soc. **59**, 1134 (1937).
[9] Bernal and Fowler, J. Chem. Phys. **1**, 515 (1933).
[10] Errera and Mollet, Nature **138**, 882 (1936).
[11] Kinsey and Ellis, J. Chem. Phys. **5**, 399 (1937).
[12] Ganz, Ann. d. Physik **28**, 445 (1937).
[13] Ellis, Phys. Rev. **38**, 693 (1931).
[14] Kellner, Proc. Roy. Soc. **159**, 410 (1937).
[15] Collins, Phys. Rev. **52**, 88 (1937).

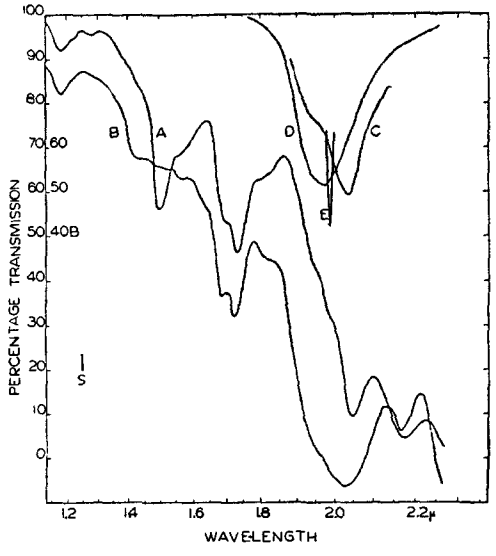

FIG. 3. A: 0.99 mm oven-dried gelatin. B: 1.49 mm gelatin+D_2O. (Percentages in B have been displaced downward 10 percent.) C: 0.30 mm gelatin+D_2O. D: 0.08 mm D_2O. E: D_2O in CCl_4.

This effect of intensification is probably responsible for the appearance for the first time of a weak band at 1.35μ, also shown in Fig. 2B. This we interpret as $(\nu_\sigma+\nu_\pi+\nu_R)$.

A similar effect is doubtless responsible for the unequal rates of descent toward the 2.6μ region in which $(\nu_\sigma+\nu_R)$ is located.

The irregularity in Fig. 2B at 1.7μ is caused by the nonsuperposition of C−H bands in this region. The slight shift in the C−H bands producing this irregularity is clearly shown in Fig. 1I–J, representing the effects of bound heavy water, and will be discussed more fully later. Peculiarities of absorption in the 2.2–2.3μ region will also be discussed later.

It was our opinion, after inspecting Fig. 2B, that we were wrong in our original assumption that the gelatin bands would not be greatly altered upon saturating the specimen with water. We conclude that the 1.50μ and 2.05μ N−H bands diminished appreciably in intensity, thus falsifying in Fig. 2B the shapes of the two water bands which overlap these N−H regions. Guided by Fig. 2A, we have drawn with broken lines the probable contours that would appear if there were no falsification. We thus estimate that the 1.50μ and 2.05μ NH bands have diminished to at least one-half and three-fourths of their original intensities, respectively.

To test the preceding assumption we repeated the experiments outlined above, using 99.9 percent heavy water whose absorption bands do not overlap the 1.44μ region. During the first attempt it was found that condensation of heavy water was occurring on the glass plates between which the gelatin specimens were sealed. This was caused by an air jet which, as in the previous experiments, was used to keep the temperature from rising. Consequently, the air jet had to be dispensed with and the plates dried and resealed. The spectrum showed the presence of light water. Replacement of H atoms of the protein by D atoms from the heavy water was suspected. But the probability of this having happened was removed when, upon drying the specimen, it was found to yield the same N−H and C−H bands and no new ones. The experiment was again repeated with every precaution taken to prevent contamination with light water vapor. The record, showing no light water absorption at 1.44μ and perhaps none at 1.93μ, is shown in Fig. 1H. A dry specimen record is given in Fig. 1G for comparison.

The suspicion of falsification of the absorption bands of Fig. 2B is now corroborated. For, with heavy water the 1.50μ N−H band is completely removed and the 2.05μ and 2.18μ N−H bands are greatly reduced in intensity. It is also apparent from Fig. 1G–H that there is a continuous absorption, extending from the 2.05μ band to 2.42μ ,which characterizes the oven-dried gelatin spectrum. This continuous absorption is greatly diminished in the spectrum of the water-bound specimen.

To demonstrate further the effects referred to in the preceding paragraph, the experiment was repeated with specimens which were five times as thick as before. These were made by immersing ten sheets of gelatin in a mixture of CCl_4 and CS_2. Thus by matching the index of refraction of the mixture with that of the gelatin, it was possible to make a fairly transparent specimen. CCl_4 and CS_2 are the two common liquids which do not have absorption bands of their own in the region studied. They mix readily and fortunately have indices below and above that of gelatin, respectively. The records for 0.99 mm oven-dried and 1.49 mm D_2O saturated specimens are shown in Fig. 1I–J.

The records of Fig. 1I–J have been reduced to percentage transmission curves, using an appropriate background record. These curves are plotted in Fig. 3A–B. The deep 1.50μ N−H valence band of Fig. 3A is nearly, or possibly entirely, gone in Fig. 3B. Because of the appearance of a complicated structure in the N−H valence bands of primary and secondary amines, it is impossible to say if the long wave shoulder of this band is to be attributed to N−H valence oscillators which are more highly perturbed than those contributing to the deeper short wave portion of the band. That there is such an effect in aniline, a primary amine, has been shown by Kinsey and Ellis [11] through the disappearance of the long wave portion of the 1.5μ band when aniline is dissolved in CCl_4. A search for a similar effect in a secondary amine, di-*n*-butylamine, during the present investigation led to negative results. All, or most, of the gelatin N−H oscillators contributing to the 1.50μ gelatin band must be perturbed by hydrogen bridging according to the model of Astbury and Lomax.[16] The bridges should occur between the N−H groups of one molecule and the C=O groups of another.

The surprising thing in connection with the 1.50μ band is its partial disappearance when H_2O molecules are attached to the N−H groups and its almost complete disappearance when D_2O molecules are attached. It is not likely, in view of the relative strengths of ordinary chemical bonds and hydrogen bridges, and also in view of the nature of the spectrum resulting when a D_2O swollen specimen had been carefully re-dried, that the N−H groups are actually destroyed by the addition of water. It is more likely that the dipole moments in the N−H groups have been greatly reduced when the water molecules are bridged to them, and that D_2O is much more effective than H_2O. Also it is possible that the C=O···H−N bridging enhances the intensity of the band of the dried gelatin. This possibility is suggested by the results of Gordy[17] which show that the intensities of the N−H fundamental valence bands of aniline and the C=O fundamental valence band of acetone are both enhanced when these two liquids are mixed. This effect, as Gordy suggests, probably results from C=O···H−N bridging. A more startling, but less analogous, example of such an intensity alteration is found in the 100-fold increase of absorption by H_2O molecules in solutions of dioxane or pyridine[18] over the absorption that they possess in the inactive solvents CCl_4 and CS_2. It should be pointed out in this connection that Fig. 2A–B shows that the total absorption by water molecules is not increased when these are bound to gelatin. If the preceding analysis is correct, a cause for the opposite effects produced by C=O and D_2O (and H_2O) may be that in the former instance the N−H group is the donor of the H for the bridge, whereas in the latter instance it may be the acceptor as well as the donor.

The 2.05μ and 2.18μ N−H bands behave similarly to the 1.50μ band, but to a lesser degree. Fig. 1G–H clearly shows that these bands have intensities diminished when D_2O is added. That there is a similar but smaller effect with H_2O is seen from the appearance of Fig. 2B in the regions 2.05μ and 2.18μ, Only a small portion of this diminution could be caused by the 6 percent increase in area of the gelatin specimen when the water is taken on. The difference in the behavior of the 1.50μ and the 2.05μ, 2.18μ bands may be caused by a smaller alteration of the dipole moment change perpendicular to the N−H groups than parallel to the groups. The oscillations producing the 2.05μ and 2.18μ bands have transverse components.

The best explanation for the presence of continuous absorption in the 2.18–2.42μ region of the spectrum of the dried gelatin is that in this region there are long wave contributions to the 2.05μ and 2.18μ bands similar to the shoulder of the 1.50μ band. These long wave contributions may arise from the more highly perturbed N−H or NH_2 groups. Variations in the strength of the hydrogen bridge may be caused by the frequent occurrence of proline linkages in the backbone. These proline linkages probably cause a warping in the backbone, resulting in closer approaches of some C=O and N−H groups than of others. It is possible that the investigation of silk, in which there are fewer proline linkages, will give additional information in this respect.

[16] Astbury, Trans. Faraday Soc. **29**, 193 (1933); Astbury and Lomax, J. Chem. Soc. London, p. 846, June, 1935.
[17] Gordy, J. Am. Chem. Soc. **59**, 464 (1937).
[18] Errera, Physica **4**, 1097 (1937).

Figure 3A shows a weak band at 1.28μ and a very weak one at 1.22μ which disappear in Fig. 3B. These bands doubtless bear the same relation to the 1.50μ overtone as the 2.18μ and 2.05μ bands bear to the valence vibration fundamental near 3.0μ.

The 1.18μ, 1.72μ and 2.28μ bands arise from C−H vibrations. The most conspicuous of these, namely the one at 1.72μ, shifts toward shorter wave-lengths upon the addition of water, as is clearly shown by Figs. 1I–J and 3A–B. This is to be expected since the C−H groups contributing to it have fewer near neighboring atoms when the gelatin molecules are pushed apart by water molecules. No bridging of water molecules to C−H groups is probable. This shift toward shorter wave-lengths when conditions are more vapor-like for the C−H groups is consistent with general observations of differences in band positions in vapor, liquid and solution spectra.[19] Some of the change of form in the 1.72μ band is doubtless significant. However, it is also partly caused by a partially overlapping D_2O band.

Weak absorption bands at 1.94μ and 1.99μ in Figs. 1I and 3A may well be attributed to carbonyl groups C=O.[20]

The main band of D_2O occurs at 1.98μ. This is $(\nu_\sigma+\nu_\pi)$. Its position in Fig. 3B is certainly too far to the right, owing to the overlapping of a residual of the 2.18μ N−H band. This band is also shown in Fig. 3C, which is a similar reduction of the record of Fig. 1H. Fig. 3B–C shows a short wave-length shoulder in this band. This absorption and that at 1.44μ in Fig. 3B indicate that there has been slight contamination by light water. That the short wave portion of the 1.98μ band is not entirely caused by light water impurity is shown by Fig. 3D. This represents a reduction of the record for the 0.08 mm sample of 99.9 percent D_2O shown in Fig. 1F. This record shows no impurity band at 1.44μ. The additional 1.9μ contribution can arise from $(\nu_\sigma+\nu_\pi+[\nu_T, \nu_R])$ and is analogous to $(\nu_\delta+[\nu_T, \nu_R])$ found by Ellis and Sorge[21] at 6.85μ. Here ν_T and ν_R are the frequencies of hindered translation and hindered rotation. That we are dealing with the weighted unresolved mean $[\nu_T, \nu_R]$ of these two frequencies follows from Cartwright's[22] measurements of the magnitudes of these frequencies, 160 cm^{-1} and 360 cm^{-1}, respectively. The center of the broad 6.85μ is separated by 240 cm^{-1} from the 8.20μ(ν_δ) band. It was pointed out by Ellis and Sorge that the extramolecular absorption contributing to the 6.85μ band and to the 1.56μ band $(\nu_\sigma+\nu_\pi+\nu_\delta+[\nu_T, \nu_R])$ is more intense than that contributing to corresponding bands in light water. The 1.56μ band is clearly shown in Figs. 1H and 3B. Also the 1.65μ band $(\nu_\sigma+\nu_\pi+\nu_\delta)$ associated with the 1.56μ is seen best in Fig. 1H.

In Fig. 3E is drawn a portion of the partially resolved $(\nu_\sigma+\nu_\pi)$ band of D_2O in CCl_4 solution.[7] Because of the similarity between this and the corresponding bands of Fig. 2C we identify this as the unresolved Q-branch of D_2O vapor-like molecules. That a considerable portion of the liquid band lies to the left of this sharp 1.98μ band, in contrast to the H_2O band behavior at 1.44μ, indicates again that there is an additional contribution in this instance.

[19] Ellis and Kinsey, J. Chem. Phys., **6**, 497 (1938).
[20] Ellis, J. Am. Chem. Soc. **51**, 1384 (1929).
[21] Ellis and Sorge, J. Chem. Phys. **2**, 559 (1934).
[22] Cartwright, Phys. Rev. **49**, 470 (1936).