EXPERIMENTAL AND MOLECULAR PATHOLOGY 55, 269–284 (1991)

Distinctive Infrared Spectral Features in Liver Tumor Tissue of Mice: Evidence of Structural Modifications at the Molecular Level[1]

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Received June 18, 1991, and in revised form July 30, 1991

Mice were treated with griseofulvin (GF) containing diet or control diet for 12 months. The livers from mice fed griseofulvin showed large tumors that were excised and used for analysis. The infrared spectra from control liver tissue and tumor tissue from GF livers were measured and compared as a function of pressure up to 27 kbar. Many changes in the infrared spectral features of the tumor tissue were observed. Results showed that neoplasm formation involved structural modifications of nucleic acids, lipids, carbohydrates, and proteins in the liver cells, which were detected from the abnormal vibrations of the functional groups in these biomolecules. The amount of glycogen was dramatically decreased in the tumor tissue compared to the control tissue. Important changes in the strength of hydrogen-bondings in the phosphodiester backbone of the nucleic acids and in the C–O groups of tissue proteins and carbohydrates were observed. Stronger interchain interactions and thus close interchain packing among the lipids in the GF liver were evident. These results showed very close similarities with those obtained with other types of tumors such as human colon cancer, suggesting that a common pattern of molecular changes has been identified in neoplastic transformation.

INTRODUCTION

During the process of tumor development in the liver, a number of changes develop in liver cell morphology, although the molecular composition of the constituents in the tissues does not alter significantly (Koen *et al.*, 1983). Therefore, molecular rearrangements and thus structural changes at the molecular level are expected in these liver cells. The phenotypic changes have been studied by several methods: light microscopy, electron microscopy, enzyme histochemistry, immunohistology, biochemical study, and molecular biology analysis (Koen *et al.*, 1983; Campbell *et al.*, 1986; Sell *et al.*, 1987; Faber and Sarma, 1987; Tournier *et al.*, 1988). However, little structural information concerning the molecular constituents has been found by these studies.

Infrared spectra of biological systems originate from vibrational modes of various molecular functional groups in these systems. Features in these spectra are strongly affected by the intra- and intermolecular interactions and thus they are structural dependent. When an external pressure is applied, these intra- and intermolecular interactions are dramatically enhanced. Consequently, the effects of these interactions on the infrared spectra are magnified. Fourier-transform infrared spectroscopy combined with the use of pressure (pressure-tuning Fourier-transform infrared spectroscopy) has been shown to be a powerful tool for the study of structural and dynamic properties in model biological systems (Wong,

[1] Issued as NRCC No. 32288.

0014-4800/91 $3.00

1987a,b,c; Wong *et al.*, 1988, 1989; Wong and Heremans, 1988; Wong and Huang, 1989; Carrier *et al.*, 1990a,b; Zakim and Wong, 1990). In order to study changes in molecular structure in biological tissues, it is necessary to obtain pressure-tuning infrared spectra of intact tissue samples instead of those of isolated constituents from the tissue, since the structural properties of cellular components in the isolated state are no longer the same as those in the intact tissue. The application of the pressure-tuning infrared spectroscopic technique to the study of tissue samples has been hampered because of the strong infrared absorption by tissue water and difficulties with sample preparation for high quality infrared spectra. These methodological problems have been overcome recently in our NRCC laboratory (Wong, 1990a,b; Wong and Rigas, 1990) and spectra of extremely high quality with undetectable noise for tissue samples are now obtained routinely. This development has made possible the use of pressure-tuning Fourier-transform infrared spectroscopy for the study of structural properties of cellular components within intact tissue (Wong and Rigas, 1990; Auger *et al.*, 1987; Takahashi *et al.*, 1989, 1990; Rigas *et al.*, 1990). In the present study, we analyzed the modifications in the infrared spectral features and thus the changes in the molecular structure that are involved in liver tumor formation in mice.

For studying liver tumor transformation we have used the griseofulvin (GF)-fed mouse model. GF is an antifugal agent that blocks mitosis by inhibiting microtubule polymerization. GF treatment of mice is also associated with a complex liver pathology corresponding to alteration of porphyrin metabolism and, after long-term feeding, cholestasis, disturbance of the arrangement of intermediate filaments, and neoplastic transformation. Large neoplastic nodules are induced by feeding mice with GF continuously for 10 months (Denk *et al.*, 1984; and Tazawa *et al.*, 1983). Morphological studies on these livers (Tazawa *et al.*, 1983) have shown that most parts of the tumor were hyperplastic nodules and that less than 10% were hepatoma. In the present article we compared the infrared spectra from the control liver and liver tumors from GF-treated mice.

EXPERIMENTAL

Adult C3H mice were pair fed a semisynthetic complete diet with or without 2.5% (w/w) griseofulvin (gift of Schering Corp., Bloomfield, NJ) for 12 months according to the method of Denk *et al.* (1975, 1979). The livers of the GF mice and control mice were removed under anesthesia with pentobarbital. Control liver was cut into small pieces, frozen in isopentane, precooled in liquid nitrogen, and stored at -80°C until used. Tumors from GF livers were excised and processed in the same manner. We have systematically studied the stability of the tissue samples at various temperatures and found that at temperatures below 12°C, the tissue samples are very stable and no change in the spectra was observed for at least a few days.

Small amounts (about 0.01 mg) of tissue samples were placed at room temperature together with powdered α-quartz in a 0.37-mm-diameter hole in a 0.23-mm thick stainless steel gasket mounted on a diamond anvil cell. Pressures on the samples were determined from frequency shifts of the 695 cm^{-1} phonon band of α-quartz (Wong *et al.*, 1985). The pressure was raised up to 27 kbar while spectra were taken at each increment.

Infrared spectra at various pressures were obtained with a Digilab FTS-60 Fourier transform spectrometer using a liquid nitrogen-cooled mercury cadmium

telluride detector. For each spectrum, 512 interferograms were co-added at a spectral resolution of 4 cm^{-1}. Data reduction was performed using software developed in the NRCC laboratory.

RESULTS AND DISCUSSION

Infrared spectra of liver tissue from mice pair fed with a control diet or GF-containing diet for 12 months have been measured as a function of pressure up to 27 kbar. The results presented here are reproducible among samples from three pairs of mice. Fig. 1 shows the representative infrared spectra of control mouse liver tissue and tumor tissue from GF-treated mouse liver in the frequency region 950–1350 cm^{-1}. Dramatic changes in the intensity and band shape between the control and the GF livers are observed in this frequency region. Figures 2 and 3 show the infrared spectra of the same liver samples in the frequency regions 1350–1800 and 2800–3050 cm^{-1}, respectively. Some changes in band intensity are also observed in these frequency regions.

Infrared band assignment. As evident from Figs. 1–3, the infrared spectrum of liver tissue is not as complicated as was anticipated. Moreover, infrared spectra of tissues of various biological organs obtained in our laboratory (Wong and Rigas, 1990; Rigas *et al.*, 1990; unpublished work) show that the main features in these spectra are about the same and these spectra consist of absorption bands similar to those in the infrared spectrum of a mixture of proteins, nucleic acids, lipids, and carbohydrates which are the main constituents in biological tissues (Sheeler and Bianchi, 1980). Therefore, the observed bands in the infrared spectrum of liver tissues are due to the vibrational modes of the functional groups in the molecules of these constituents in liver tissues. Detailed assignments of the infrared bands in the spectra of various tissue samples have been done by comparison of the infrared spectra of proteins, lipids, nucleic acids, and carbohydrates isolated from tissues and cultured cells (Wong *et al.*, 1991; unpublished work).

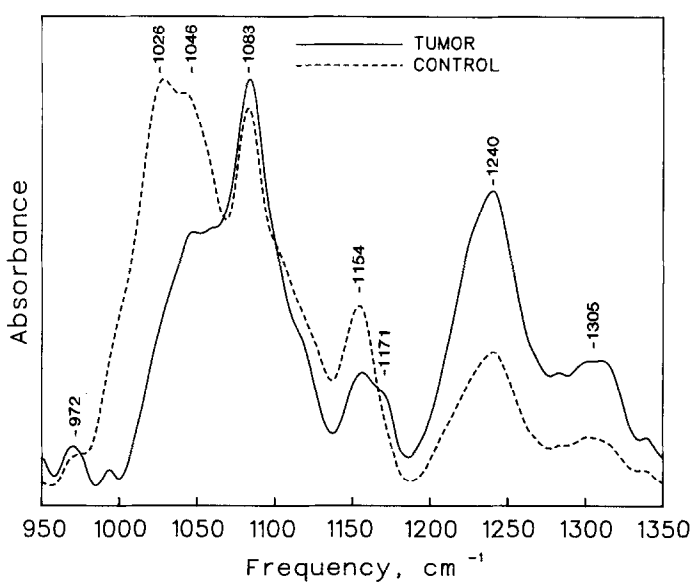

FIG. 1. Infrared absorption spectra of tissue sections from the GF and the control livers in mice in the frequency region 950–1350 cm^{-1}; auto-scale plotting.

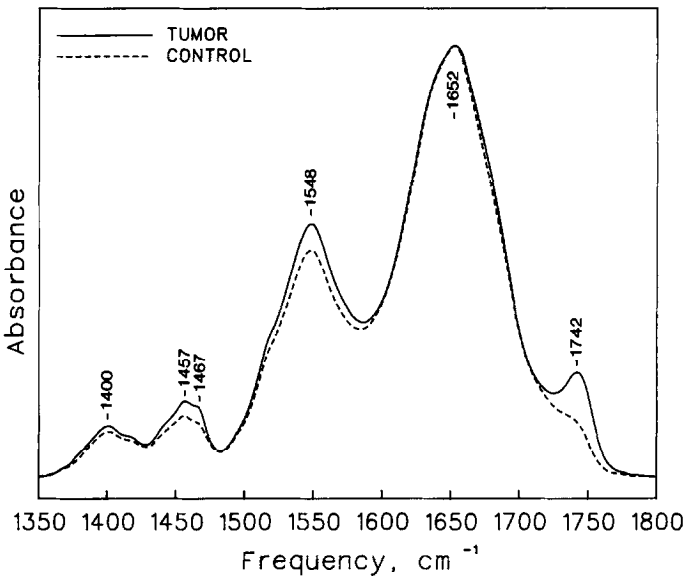

FIG. 2. Infrared absorption spectra of tissue sections from the GF and the control livers in mice in the frequency region 1350–1800 cm^{-1}.

Since the assignments of the infrared bands in the individual spectrum of proteins, lipids, nucleic acids, or carbohydrates are well established (Parker, 1971, 1983, and references therein), by comparison between the infrared spectra of tissue samples and those of the individual components, we are able to identify the infrared bands specifically from proteins, lipids, nucleic acids, or carbohydrates and the magnitude of influence among them in the spectra of tissues. For instance, the bands at 1083 and 1240 cm^{-1} are due to the symmetric ($\nu_s PO_2^-$) and anti-

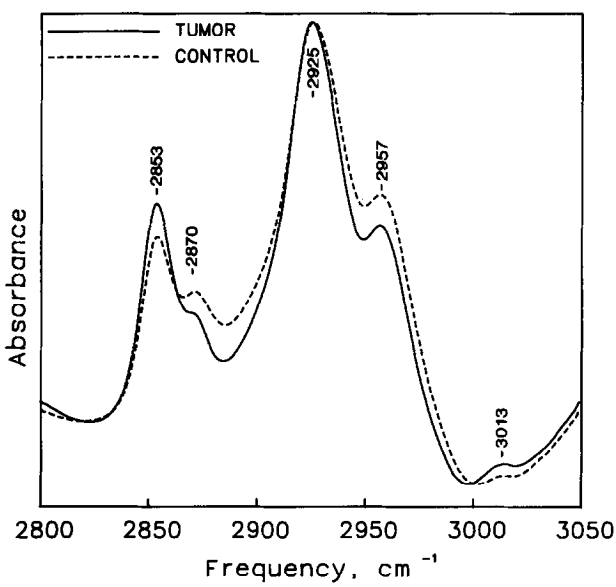

FIG. 3. Infrared absorption spectra of tissue sections from the GF and the control livers in mice in the frequency region 2800–3050 cm^{-1}; auto-scale plotting.

symmetric ($\nu_{as}PO_2^-$) PO_2^- stretching modes, respectively (Parker, 1971, 1983). PO_2^- groups are present in both nucleic acids and phospholipids. However, the peak intensity ratio between the C=O stretching band at ~1740 cm^{-1} and the $\nu_{as}PO_2^-$ band at 1240 cm^{-1} of phospholipids is in the range of 1.9–2.3, (Wong *et al.*, 1991), and on the other hand, this peak intensity ratio in the spectra of control liver tissue is only 0.14–0.34. Therefore, the contribution to the intensity of the 1240 cm^{-1} band in the spectra of liver tissue by phospholipids is negligible and the $\nu_{as}PO_2^-$ band as well as the $\nu_sPO_2^-$ band in the infrared spectra of liver tissue are mainly due to the vibrations of the PO_2^- groups in nucleic acids. The amount of other compounds containing phosphodiester groups in the liver tissue is much smaller than that of phospholipids (Sheeler and Bianchi, 1980). Consequently, their contributions to the intensities of these phosphate bands in the infrared spectra of liver tissue are also negligible.

The amide III band of proteins may also contribute to the intensity in the frequency region of the antisymmetric PO_2^- stretching band. However, its frequency varies according to the secondary structure of proteins. The secondary structure of proteins can be determined by the frequency of the amide I band (Parker, 1971, 1983; Susi, 1969). As seen in Fig. 2, the peak positions of the amide I bands of liver tissue proteins in both control liver and liver tumor are at 1652 cm^{-1}, which indicates that the secondary structure of the proteins in both tissue samples is mainly α-helix (details are given later). The amide III band of proteins with α-helical structure is located in the frequency region 1260–1290 cm^{-1} (Bandekar and Krimm, 1979), which is much higher than the frequency of the $\nu_{as}PO_2^-$ band. Consequently, the amide III band of the tissue proteins does not contribute to the intensity of the 1240 cm^{-1} band in the spectra of liver tissues of mice. The infrared spectra of isolated proteins from the control liver and liver tumor tissues also show that the amide III band does not contribute to the intensity of the band at 1240 cm^{-1} (to be reported elsewhere).

The assignments of the two phosphate bands in the spectra of liver tissues to the vibrational modes of PO_2^- groups in nucleic acids in the liver tissue are consistent with those given in the literature (Benedetti *et al.*, 1990; Woernley, 1952). Recently, the study of infrared spectra of tissue cells and the nuclei DNA and RNA isolated from these cells confirms this assignment (Wong *et al.*, 1991).

The band at 1154–1171 cm^{-1} is due to the C–O stretching (νC–O) of the COH group in serine, threonine, and tyrosine residues of cell proteins as well as in carbohydrates (Parker, 1971, 1983). The contribution to this band by the C–O groups in the acyl chains of membrane lipids is negligible, as indicated by the relative intensities of the C–O and the C=O stretching modes of membrane lipids. The peak intensity ratio between the C=O stretching band and the C–O stretching band of lipids is in the range 5.2–12.4 (Wong *et al.*, 1991), whereas in the spectra of liver tissue this ratio is 0.6–0.8. Apparently, the C–O stretching band in the spectra of liver tissues is essentially from the C–O stretching mode of tissue proteins and carbohydrates.

Other bands in the infrared spectra of liver tissues can be assigned in the same way as follows. Several overlapping bands in the frequency region 1000–1080 cm^{-1} in the spectra of liver tissues (Fig. 1) are mainly due to the vibrational modes of the CH_2OH groups and the C–O stretching coupled with the C–O bending of the C–OH groups in carbohydrates. The infrared spectrum of glycogen (D-glycogen from mammalian liver, Aldrich, Milwaukee, WI) is essentially superim-

posable with that in Fig. 1 in the frequency region 975–1080 cm^{-1} (unpublished observation). The vibrational modes of other carbohydrates also contribute slightly to the intensities of these overlapping bands.

In Fig. 2, the two strongest bands at 1652 and 1548 cm^{-1} in the spectra of liver tissue are mainly due to the amide I and amide II bands, respectively, of the amide group vibrations in tissue proteins. The bands at ~1400 and ~1457 cm^{-1} mainly arise from the symmetric ($\delta_s CH_3$) and asymmetric ($\delta_{as} CH_3$) CH_3 bending modes, respectively, of methyl groups in tissue proteins. The CH_2 bending mode (δCH_2) and the C=O stretching ($\nu C=O$) modes of the acyl chains in lipids are located at 1467 and 1742 cm^{-1}, respectively. The bands at 2853 and 3013 cm^{-1} in Fig. 3 are due to the symmetric CH_2 stretching mode of the methylene chains and the olefinic CH stretching mode in lipids, respectively (Parker, 1971, 1983; Fringeli and Gunthard, 1981). The assignment of these infrared bands in the spectra of liver tissues are summarized in Table I.

Glycogen bands. The most dramatic change in the spectrum of liver tumor tissue is the decrease in the intensities of the glycogen bands in the frequency region 1000–1050 cm^{-1} and at 1154 cm^{-1}. These changes in the spectrum are consistent with the well-known fact that in neoplastic liver cells, the glycogen level decreases dramatically, which leads to hypoglycemia (Edmondson and Peters, 1977).

The C – O stretching band. The center of gravity of the band at 1154 cm^{-1} in the spectrum of control liver shifts to about 1164 cm^{-1} in liver tumor and changes in the band shape are also observed. These bands from the control and the GF liver are enlarged and superimposed in Fig. 4. Fig. 4A shows the original spectra, whereas Fig 4B shows the corresponding $\nu C-O$ spectra after band narrowing using Fourier self-deconvolution with enhancement factor of 1.4 and band width of 20 cm^{-1} (Cameron and Moffatt, 1984). It is clear in Fig. 4 that this $\nu C-O$ band splits into two at 1154 and 1171 cm^{-1} in the deconvolved spectra. The intensity of the 1171 cm^{-1} band is very low in the spectrum of the control liver and increases at the expense of the 1154 cm^{-1} component band in the spectrum of the GF tumor

TABLE I
Frequency (in cm^{-1}) and Assignment of the Major Vibrational Modes in the Infrared Spectrum of Liver Tissue

Frequency, cm^{-1}	Vibrational mode	Major contribution
3013	ν=CH	Lipids
2957	$\nu_{as}CH_3$	Lipids & proteins
2925	ν_{as}CH2	Lipids
2853	νsCH_2	Lipids
1742	$\nu C=O$	Lipids
1652	Amide I	Proteins
1548	Amide II	Proteins
1467	δCH2	Lipids
1457	$\delta_{as}CH_3$	Proteins
1400	$\delta_s Ch_3$	Proteins
1240	$\nu_{as}PO_2^-$	Nucleic acids
1154–1171	$\nu C-O$	Glycogen & proteins
1083	$\nu_s PO_2^-$	Nucleic acids
1046	$\nu C-O + \delta C-O$	Glycogen
1028	νCH_2OH	Glycogen

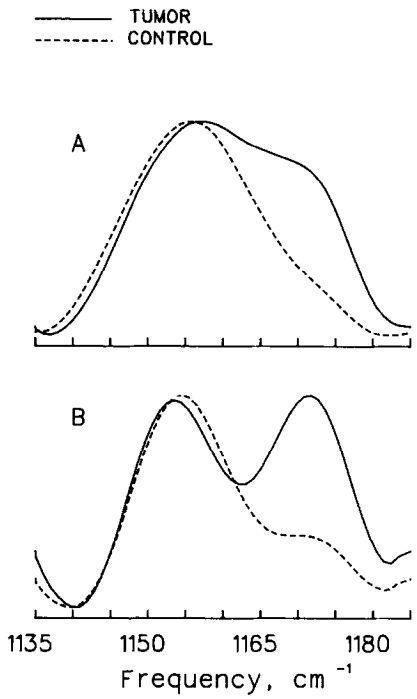

FIG. 4. Infrared absorption spectra of a pair of control and GF live tissues in the frequency region 1135–1185 cm^{-1}; auto-scale plotting. (A) original spectra, (B) spectra after band narrowing using Fourier self-deconvolution as in text.

liver. The pressure dependences of the frequencies of these two component bands of a GF liver tissue are shown in Fig. 5. The frequency of the 1171 cm^{-1} band increases while that of the 1154 cm^{-1} band decreases with increasing pressure. These frequencies were calculated from the third order derivative spectra with a breakpoint of 0.4 (Cameron and Moffatt, 1984). In general, the pressure-induced

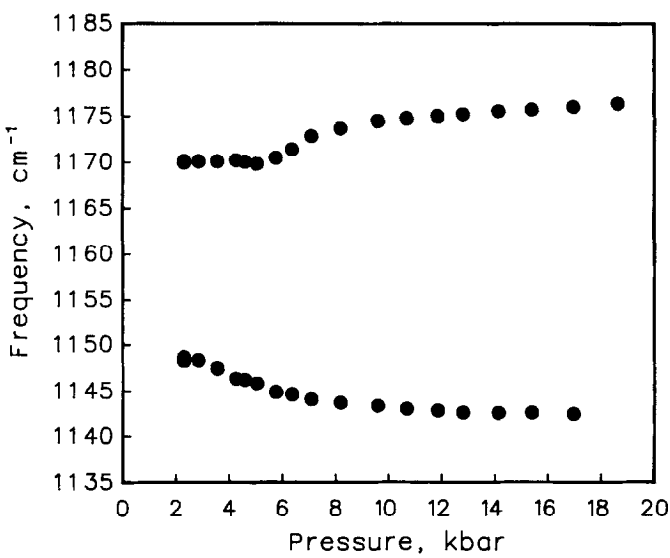

FIG. 5. Pressure dependencies of the C–O stretching frequencies of the GF liver tissue.

increase in the $\nu C-O$ frequency is the result of the compression of the C – O bond and the pressure-enhanced intermolecular interactions (Wong, 1987a). On the other hand, if the C – O group is hydrogen-bonded, the $\nu C-O$ frequency is decreased and is decreased further with increasing pressure (Wong, 1987a; Rigas *et al.*, 1990). Therefore, the 1154 cm^{-1} band is due to the $\nu C-O$ mode of the hydrogen-bonded C – O groups whereas the 1171 cm^{-1} band is due to the stretching of the nonhydrogen-bonded C – O groups. The increase in the intensity of the 1171 cm^{-1} band and the decrease in the intensity of the 1154 cm^{-1} band in the spectrum of the GF liver tumor indicates that more such C – O groups become nonhydrogen-bonded in the GF liver. Similar changes in the $\nu C-O$ band have been observed in human malignant colonic tissues (Rigas *et al.*, 1990). However, in the malignant colonic tissue, the change in the relative intensities of these two C – O bands is more dramatic and the low-frequency C – O band is almost disappeared in the malignant tissue. It has been shown (Denk *et al.*, 1982) that the phosphorylated form of some proteins are more predominant in the GF liver compared to the control one. When the OH atoms of the COH groups in a protein molecule are replaced by phosphate groups in phosphorylation, these C – O groups become more difficult to form hydrogen bonds with other molecules because of the steric-hindered effect of the phosphate groups. This could be the reason that the number of nonhydrogen-bonded C – O groups is increased in the GF tumor liver proteins.

Phosphate stretching bands. Fig. 6A shows the infrared bands of the $\nu_{as}PO_2^-$ mode of the nucleic acid backbone in a control compared with GF liver tissue. Fig. 6B shows the corresponding deconvolved spectra with an enhancement fac-

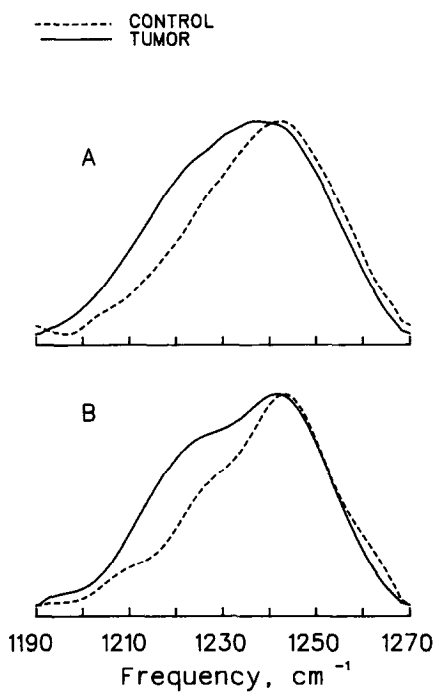

FIG. 6. Infrared absorption spectra of a pair of control and GF live tissues in the frequency region 1190–1270 cm^{-1}; auto-scale plotting. (A) original spectra, (B) spectra after band narrowing using Fourier self-deconvolution as in text.

tor of 1.4 and a band width of 20 cm^{-1} (Cameron and Moffatt, 1984). It is evident from Fig. 6 that this $\nu_{as}PO_2^-$ band also consists of two component bands at 1240 and 1224 cm^{-1}. The intensity of the 1224 cm^{-1} band is weak in the control liver whereas it is increased considerably in the GF liver. The $\nu_{as}PO_2^-$ band is at about 1220 cm^{-1} for a fully hydrated PO_2^- group and is at above 1240 cm^{-1} for an anhydrous PO_2^- group (Susi, 1969). The increase in the relative intensity of the 1224 cm^{-1} band in the GF liver tumor indicates that the number of hydrated phosphate groups is increased in the nucleic acids of the GF liver tissue. The pressure dependencies of the frequencies of the $\nu_{as}PO_2^-$ component bands are shown in Fig. 7 which were calculated from the third power derivative spectra with a breakpoint of 0.3 (Cameron and Moffatt, 1984). The 1224 cm^{-1} band frequency decreases with increasing pressure and then levels off at high pressure, which confirms that this band is due to the $\nu_{as}PO_2^-$ mode of hydrogen-bonded PO_2^- groups with water (Wong, 1987a; Wong and Mantsch, 1988). The increase in the intensity of the hydrated PO_2^- stretching band at 1224 cm^{-1} has also been observed in the infrared spectrum of human malignant colonic tissues (Rigas *et al.*, 1990). The increase in the intensity of the hydrated PO_2^- stretching band is much larger in the spectra of malignant colonic tissues.

Fig. 8 shows the pressure dependencies of the symmetric stretching frequencies ($\nu_sPO_2^-$) of the phosphate backbone in the nucleic acids of a control compared with a GF liver. These frequencies were obtained from the third power derivative spectra with a breakpoint of 0.4 (Cameron and Moffatt, 1984). The $\nu_sPO_2^-$ frequency increases with increasing pressure for both the control and the GF liver and it is about 3 cm^{-1} higher in the GF tissue at all pressures (Fig. 8). The same results for the $\nu_sPO_2^-$ frequency have been observed between the normal and malignant human colonic tissue (Wong and Rigas, 1990; Rigas *et al.*, 1990). The increase in the $\nu_2PO_2^-$ frequency in the GF tissue compared with that in the control tissue suggests that the structure of the phosphate backbone of the nucleic

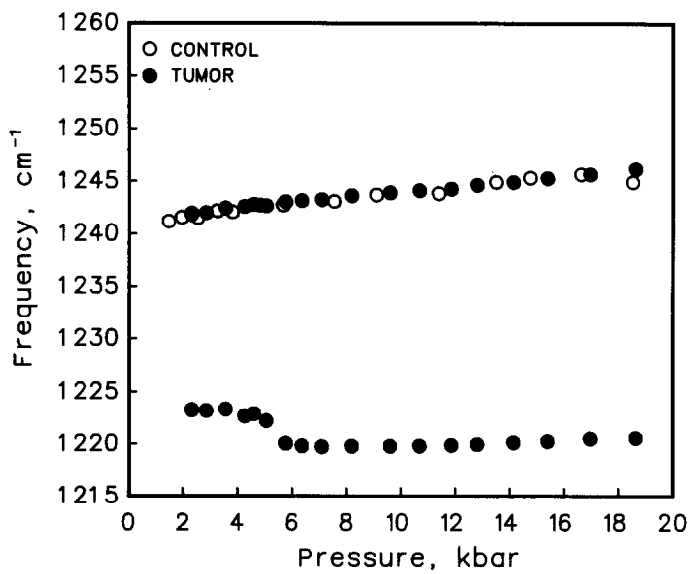

FIG. 7. Pressure dependencies of the antisymmetric phosphate stretching frequencies of the control and the GF liver tissues.

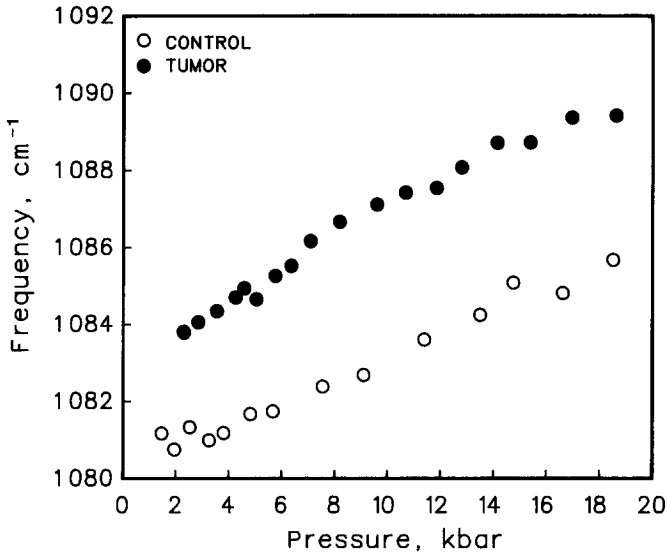

FIG. 8. Pressure dependencies of the symmetric phosphate stretching frequencies of the control and the GF liver tissues.

acids in the GF tissue is changed. In general, the normal mode frequencies of a functional group of long chain systems would increase when the interchain interactions are enhanced (Wong, 1987a; Wong and Zakim, 1990). The increase in the $\nu_s PO_2^-$ frequency in the GF tissue may be the result of the stronger interchain interactions in the nucleic acids of the GF tissue due to a closer packing between neighboring nucleic acid molecules. The increase in the number of hydrogen-bonded PO_2^- groups in the GF tissue as observed in the $\nu_{sa}PO_2^-$ band in Fig. 6 may contribute to some intensity on the low-frequency side of the $\nu_s PO_2^-$ band (Susi, 1969).

CH stretching bands. The infrared spectra of a control and GF liver tissue in the CH stretching region (Fig. 3) show that in the GF tissue, the relative intensities of the bands at 2853 and 3013 cm^{-1} increase while that of the 2957 cm^{-1} band decreases when compared to the corresponding bands in the control tissue. The bands at 2853 cm^{-1} and 3013 cm^{-1} are due to the symmetric CH_2 stretching mode ($\nu_s CH_2$) of the methylene chains and the olefinic CH stretching mode (ν=CH), respectively, in lipids. The band at 2957 cm^{-1} is due to the asymmetric CH stretching mode of the methyl groups ($\nu_{as}CH_3$). The $\nu_{as}CH_3$ modes of both the end-methyl groups of lipids and the methyl groups on the side chains of proteins are at about the same frequency (Parker, 1971, 1983; Fringeli and Gunthard, 1981). Therefore, the $\nu_{as}(CH_3)$ modes of the methyl groups of both lipids and proteins contribute to the intensity of the band at 2957 cm^{-1}. The changes in the relative intensity of the $\nu_s CH_2$ band and the $\nu_{as}CH_3$ band, as seen in Fig. 3, indicate that the ratio of the number of methyl groups to that of methylene groups is decreased in the GF liver tissue, as compared to that in the control tissue. The intensity ratio between the 2957 cm^{-1} band and the 2853 cm^{-1} band is also decreased in the malignant colonic tissue. However, the absolute value of this intensity ratio in colonic tissues varies from patient to patient (Rigas *et al.*, 1990).

The degree of unsaturation in the lipids of the GF liver is increased slightly with

respect to that in the control tissue as shown by the increase in the intensity of the olefinic $\nu = CH_2$ band in the spectrum of the GF tissue (Fig. 3).

The pressure dependences of the $\nu_s CH_2$ frequencies of both the control and the GF liver tissue are shown in Fig. 9. The frequency of this band can be used as a measure of the magnitude of interchain interactions in lipids (Wong, 1987a; Wong and Zakim, 1990). This frequency is higher as interchain interactions are stronger. As is evident from Fig. 9, the interactions among neighboring methylene chains of lipids are stronger and thus the interchain packing is closer in the GF liver tissue.

Pressure dependence of the CH_2 bending mode. The pressure dependences of the CH_2 bending frequencies of the methylene chains in lipids are shown in Fig. 10. A discontinuous increase in this frequency at 10 kbar is observed in the GF tissue. On the other hand, the discontinuous increase in the δCH_2 frequency in the control tissue takes place at a higher pressure and a wider pressure range (8–15 kbar). The ordering of the methylene chains of membrane lipids in the GF liver at a lower pressure and a narrower pressure range indicates that the interchain interactions are stronger and the interchain packing is closer in the lipids of the GF liver cells (Wong *et al.*, 1988; Auger *et al.*, 1988). Different types of lipids are present in liver tissue and the order/disorder transition for a lipid mixture is expected to be broad (Ladbrooke and Chapman, 1969). Therefore, the abrupt transition in the GF liver indicates that the observed results of lipids in the GF tissue may be arising from a particular lipid with large quantity in the GF tissue.

Accumulation of Triacyl glycerols. The intensity of the C = O stretching band of lipids (1742 cm^{-1}) increases more than three times in the GF tissue (see Fig. 2), indicating that the accumulation of lipids was increased considerably in the GF liver. The C = O stretching frequency in the liver tissue observed in the present work is higher than those in common membrane lipids (Wong *et al.*, 1988; Wong and Mantsch, 1988). The intensity, frequency, and its pressure dependence of the C = O band in the GF tissue are very similar to those in alcoholic fatty livers in rats (Takahashi *et al.*, 1989, 1990), in which large amounts of triacyl glycerols have

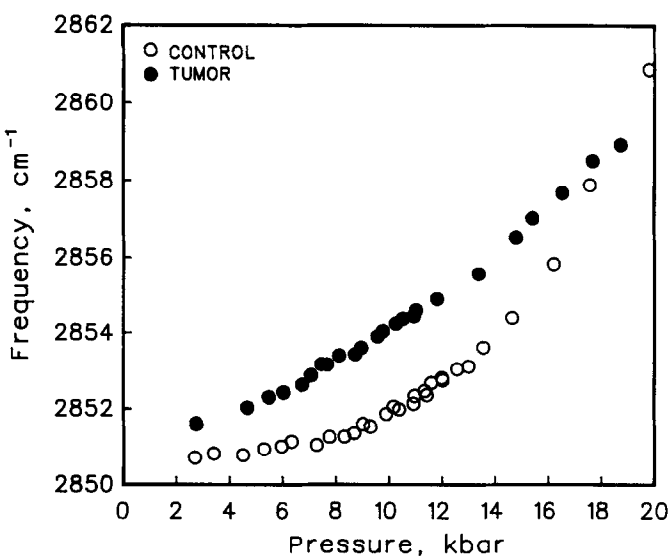

FIG. 9. Pressure dependencies of the symmetric CH_2 stretching frequencies of the control and the GF liver tissues.

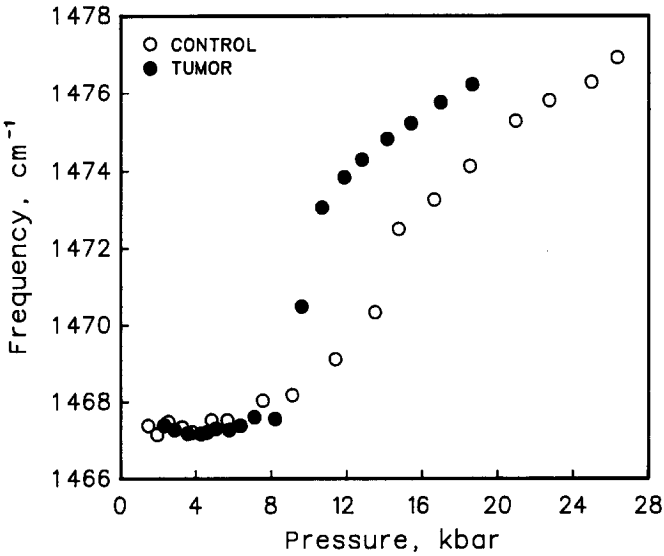

FIG. 10. Pressure dependencies of the CH_2 bending frequencies of the control and the GF liver tissues.

accumulated. Moreover, the behavior of the CH_2 bending frequency under pressure and the pattern of the pressure-induced frequency shift observed in the GF tissues (Fig. 10) resemble those of unsaturated lipid bilayers in which the conformational structure of methylene chains become highly ordered at the discontinuous shift of the δCH_2 frequency (Wong and Mantsch, 1988). Therefore, these results indicate that the accumulation of unsaturated hepatic triacyl glycerols was increased considerably in the GF liver, and the properties of the lipid methylene chains observed in the GF liver are largely originated from these accumulated triacyl glycerols.

Amide I bands. The strong band in the frequency region 1600–1700 cm^{-1} (Fig. 2) referred to as the amide I band is due to the in-plane C=O stretching vibration weakly coupled with C–N stretching and in-plane N–H bending of the amide groups in proteins (Parker, 1971, 1983; Susi, 1969). The peak maximum of the amide I band is sensitive to the secondary structure in proteins (Parker, 1971, 1983; Susi, 1969). Peak maximum is near 1650 cm^{-1} for α-helical structure and two amide I bands are present for β-sheet structures at ~1635 and ~1685 cm^{-1}. The band at ~1685 cm^{-1} is mainly due to the amide I mode of the antiparallel β-sheet. The amide I band of unordered random coils and turns are at ~1645 cm^{-1} and ~1665 cm^{-1}, respectively. Since each globular protein molecule contains segments with different substructures, the amide I band of a globular protein usually appears as a broad band with several maxima. The changes in the relative intensities of these maxima have been widely used for monitoring the changes in the secondary substructures in globular proteins (Parker, 1971, 1983; Susi, 1969).

Fig. 11 shows the pressure contour plots of the deconvolved infrared amide I bands of both the control (Fig. 11A) and the GF (Fig. 11B) tissue proteins with an enhancement factor of 1.5 and a band width of 25 cm^{-1} (Cameron and Moffatt, 1984). These spectra show that the secondary substructures in tissue proteins of both liver samples are largely α-helices with considerable segments of β-sheet (Parker, 1971; Susi, 1969). There are some differences in the secondary structure

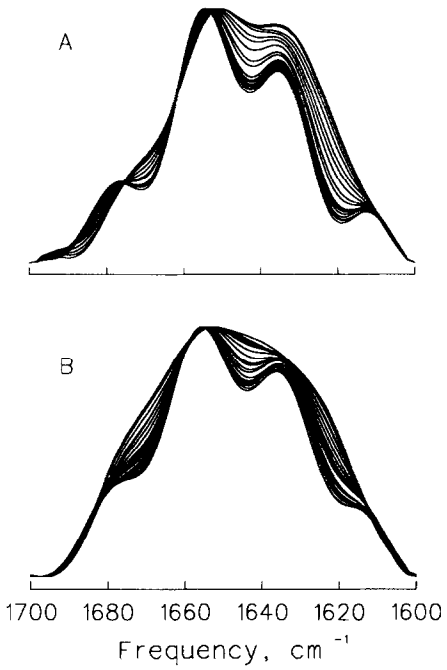

FIG. 11. Pressure contour plots of infrared absorption spectra of a pair of control (A) and GF (B) liver tissues in the amide I band region; auto-scale plotting. The spectra are obtained after band narrowing using Fourier self-deconvolution with an enhancement factor of 1.5 and a band width of 25 cm^{-1}.

between the GF and the control tissue proteins. In the GF tissue proteins, the segments of β-sheet and turns increase slightly relative to that of α-helices as indicated by the increase in the intensities near 1638 cm^{-1} and 1665 cm^{-1} in the amide I band of the GF tissue proteins. The change in the relative amounts of different conformational segments in the GF proteins may be the result of structural changes in some particular proteins which are present in both the control and the GF tissue, or alternatively the result of changes in protein composition in the GF tissue.

The pressure effects on the secondary substructures are significantly different between the GF and the control tissue proteins. As demonstrated by the pressure-induced intensity redistribution in the amide I bands, pressure induces more β-sheet than the unordered random coils and turns in the control tissue proteins (Fig. 11A), whereas it induces more unordered random coils and turns than the β-sheet in the GF tissue proteins. (Fig. 11B). Significant changes in the conformational structure start at 4.7 kbar in the control tissue proteins and 5.8 kbar in the GF tissue proteins indicating that the atmospheric substructures in the GF tissue proteins are more rigid and stable than those in the control tissue proteins.

CONCLUDING REMARKS

The present data demonstrate that griseofulvin-induced liver tumor in mice display dramatic changes in infrared spectra compared to those of the normal control mice liver. These spectral changes are the results of the alterations in the vibrational modes of the molecular functional groups in the tissues, which in turn reflect the structural and compositional changes in these important informational

and structural molecules in the tissues. The structural changes in the GF liver tissue involve the degree of hydrogen-bonding of the phosphate backbone of nucleic acids, the C – O groups in carbohydrates, and in the peptide residues of cell proteins. The number of hydrogen-bonded phosphate groups increases while that of the C – O groups decreases in the GF liver tissue. Other structural changes include a closer packing among nucleic acid chains in the GF tissue which gives rise to an increase in the phosphodiester stretching frequencies. More β-sheet and turns segments are found in tissue proteins of the GF liver as indicated by the intensity redistribution in the conformationally sensitive amide I band. The accumulation of unsaturated triacyl glycerols is increased considerably in the GF liver and the interchain interactions among these accumulated lipids are also increased in the GF liver tissue. Finally, the glycogen content decreases dramatically in the GF tissue.

The importance of dealing with intact tissues instead of isolated cellular components for the study of molecular structure of the cellular elements is also demonstrated by the present work. For instance, the nonhydrogen-bonded characteristics of the phosphate backbone of nucleic acids in the control liver tissue can be observed only when intact tissues are examined. If isolated nucleic acids are studied, this important structural information would not be obtained, since the isolated nucleic acids would certainly be hydrogen-bonded to water molecules during the wet biochemical isolation process.

The present data show clear-cut spectral differences between the control and the GF liver tissues. An important finding from the present work is that some of these spectral changes observed in the GF-induced liver tumor in mice are similar to those observed in human malignant colonic tissues (Wong and Rigas, 1990; Rigas *et al.*, 1990) and other malignant tissues (unpublished work). Consequently, the corresponding structural modifications in the cell molecules are common to both GF-induced mice liver tumors and human cancers. These findings indicate that the hyperplastic nodules induced by GF treatment have some malignancy characteristics and support the hypothesis mentioned earlier that hepatoma develop from hyperplatic nodules. The structural modifications of biomolecules in the GF liver tumor in mice, observed in the present work, and in the malignant tumor in human tissues (Wong and Rigas, 1990; Rigas *et al.*, 1990), may be related to the mechanism of neoplastic transformation in general.

The results from the present study also demonstrate that pressure-tuning infrared spectroscopy is a valuable technique for the study of cell abnormalities in biological tissues at the molecular level. Although there are many types of cells in biological tissues, the molecular constituents in biological tissues are mainly proteins, lipids, nucleic acids, and carbohydrates. Consequently, the infrared spectra of various types biological tissues are comparable and are very similar to the spectrum of a mixture of these molecular constituents and the structural changes in these constituents in abnormal cells can be easily detected by infrared spectroscopy. Our data suggest the potential applicability of this approach to a host of biological problems.

REFERENCES

AUGER, M., JARRELL, H. C., SMITH, I. C. P., SIMINOVITCH, D. J., MANTSCH, H. H., and WONG, P. T. T. (1988). Effects of the local anesthetic tetracaine on the structural and dynamic properties of lipids in model membranes: A high-pressure Fourier-transform infrared study. *Biochemistry* 27, 6086–6093.

AUGER, M., JARRELL, H. C., SMITH, I. C. P., WONG, P. T. T., SIMINOVITCH, D. J., and MANTSCH, H. H. (1987). Pressure-induced exclusion of a local anesthetic from model and nerve membranes. *Biochemistry* **26**, 8513–8516.

BANDEKAR, E., and KRIMM, S. (1979). Vibrational analysis of peptides, polypeptides, and proteins: Characteristic amide bands of b-turns. *Proc. Natl. Acad. Sci. USA* **76**, 774–777.

BENEDETTI, E., TEODORL, L., TRINCA, M. L., VERGAMINI, P., SALVATI, F., MAURO, F., and SPREMOLLA, G. (1990). A new approach to the study of human solid tumor cells by means of FT-IR microspectroscopy. *Appl. Spectrosc.* **44**, 1276–1280.

CAMERON, D. G., and MOFFATT, D. J. (1984). Deconvolution, Derivation and Smoothing of spectra using Fourier-transforms. *J. Test. Eval.* **12**, 78–85.

CAMPBELL, H. A., XU, Y. D., HANIGAN, M. H., and PITOT, H. C. (1986). Application of quantitative stereology to the evaluation of phenotypically heterogenous enzyme altered foci in rat liver. *J. Natl. Cancer Inst.* **76**, 751–767.

CARRIER, D. C., MANTSCH, H. H., and WONG, P. T. T. (1990a). Pressure-induced reversible changes in secondary structure of poly (L-lysine): An IR spectroscopic study. *Biopolymers* **29**, 837–844.

CARRIER, D. C., MANTSCH, H. H., and WONG, P. T. T. (1990b). Protective effect of lipidic surfaces against pressure-induced conformational changes of poly(L-lysine). *Biochemistry* **29**, 254–258.

DENK, H., GSCHNAIT, M.D., and WOLFF, K. (1975). Hepatocellular hyalin (mallory bodies) in long term griseofulvin-treated mice: A new experimental model for the study of hyalin formation. *Lab. Invest.* **32**, 773–776.

DENK, H., FRANKE, W. W., KERJASCHKI, D., and ECKERSTORFER, R. (1979). Mallory bodies in experimental animals and man. *Int. Rev. Exp. Pathol.* **20**, 77–120.

DENK, H., KREPLER, R., LACKINGER, E., ARTIEB, U. and FRANKE, W. W. (1982). Immunological biochemical characterization of the keratin-related component of mallory body: Pathologic pattern of hepatocytic cytokeratins. *Liver* **2**, 165–175.

DENK, H., BERNKLAU, G., and KREPLER, R. (1984). Effect of griseofulvin treatment and neoplastic transformation on transglutaminase activity in mouse liver. *Liver* **4**, 208–213.

EDMONDSON, H. A. and PETERS, R. L. (1977). Liver. *In* "Pathology" (W. A. P., Anderson, and J. M., Kissane, Eds.), Vol. 2, pp. 1321–1438. Mosby, St. Louis.

FABER, E., and SARMA, D. S. R. (1987). Biology of disease: Hepatocarcinogenesis: A dynamic cellular perspective. *Lab. Invest.* **56**, 4–22.

FRINGELI, U. P., and GUNTHARD, H. H. (1981). Infrared membrane spectroscopy. *In* "Membrane Spectroscopy" (E. Grell, Ed), pp. 270–332. Springer-Verlag, New York.

KOEN, H., PUGH, T., and GOLDFARB, S. (1983). Hepatocarcinogenesis in the mouse: Combined morphological studies. *Am. J. Pathol.* **112**, 89–100.

LADBROOKE, B. D., and CHAPMAN, D. (1969). Thermal analysis of lipids, proteins and biological membranes. *Chem. Phys. Lipids* **3**, 304–367.

PARKER, F. S. (1971). "Applications of Infrared Spectroscopy in Biochemistry, Biology and Medicine." Plenum Press, New York.

PARKER, F. S. (1983). "Applications of Infrared, Raman and Resonance Raman Spectroscopy in Biochemistry." Plenum Press, New York.

RIGAS, B., MORGELLO, S., GOLDMAN, I. S., and WONG, P. T. T. (1990). Human colorectal cancers display abnormal Fourier-transform infrared spectra. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8140–8144.

SELL, S., HUNT, J. M., KNOLL, B. J., and DUNSFORD, H. A. (1987). Cellular events during hepatocarcinogenesis and the question of premalignancy. *Adv. Cancer Res.* **48**, 37–111.

SHEELER, P., and BIANCHI, D. E. (1980). "Cellular Biology: Structure, Biochemistry and Function." 2nd ed., Wiley, New York.

SUSI, H. (1969). Infrared spectra of biological macromolecules and related systems. *In* "Structure and Stability of Biological Macromolecules" (S. N. Timsshell and C. D. Fasman, Eds.), pp. 641–659. Marcel Dekker, New York.

SUTHERLAND, G. B. B. M., and TSUBOI, M. (1957). The infrared spectrum and molecular configuration of sodium deoxyribonucleate. *Proc. Roy. Soc.* (*London*) **A239**, 446–463.

TAKAHASHI, H., FRENCH, S. W., and WONG, P. T. T. (1989). High-pressure Fourier-transform infrared spectroscopic study on molecular structures of lipids and proteins in the liver of rats chronically fed ethanol. *Hepatology* **10**, 705.

TAKAHASHI, H., FRENCH, S. W. and WONG, P. T. T. (1991). Alterations in hepatic lipids and proteins by chronic ethanol intake: A high-pressure Fourier-transform infrared spectroscopic study on alcoholic liver disease in the *Rat. Alcoh. Clin. Exp. Res.*, **15**, 219–223.

TAZAWA, J., IRIE, T., and FRENCH, S. W. (1983). Mallory body formation runs parallel to y-glutamyl transferase induction in hepatocytes of griseofulvin-fed mice, *Hepatology* **3**, 989–1001.

TOURNIER, I., LEGRES, L., SCHOEVAERT, D., FLEDMANN, G., and BERNUAU, D. (1988). Cellular analysis of D-fetoprotein gene activation during carbon tetrachloride and D-galactosamide-induced acute liver injury in rats. *Lab. Invest.* **59**, 657–665.

WOERNLEY, D. L. (1952). Infrared absorption curves for normal and neoplastic tissues and related biological substances. *Cancer Res.* **12**, 516–523.

WONG, P. T. T., MOFFATT, D. J., and BAUDAIS, F. L. (1985). Crystalline quartz as an internal pressure calibrant for high-pressure infrared spectroscopy. *Appl. Spectrosc.* **39**, 733–735.

WONG, P. T. T. (1987a). Vibrational spectra under high pressure. *In* "Vibrational Spectra and Structure" (J. R. Durig, Ed.), Vol. 16, pp. 357–445.

WONG, P. T. T. (1987b). High-pressure studies of biomembranes by vibrational spectroscopy. High-pressure chemistry and biochemistry. *NATO ASI (Adv. Sci. Inst.) Ser. Ser. C Math. Phys. Sci.* **197**, 381–400.

WONG, P. T. T. (1987c). High-pressure vibrational spectroscopy of aqueous systems: Phospholipid dispersion and proteins. *In* "Current Perspective in High Pressure Biology" (H. W. Jannash, R. E. Marquis, and A. M. Zimmerman, Eds.), pp. 287–314. Academic Press, London.

WONG, P. T. T., SIMINOVITSCH, D. J., and MANTSCH, H. H. (1988). Structure and properties of model membranes: New knowledge from high-pressure vibrational spectroscopy. *Biochim. Biophys. Acta.* **947**, 139–171.

WONG, P. T. T., and HEREMANS, K. (1988). Pressure effects on protein secondary structure and hydrogen deuterium exchange in chymotrypsinogen: A Fourier-transform infrared spectroscopic study. *Biochim. Biophys. Acta.* **956**, 1–9.

WONG, P. T. T., and MANTSCH, H. H. (1988a). Reorientational and conformational ordering processes at elevated pressures in 1,2-dioleoyl phosphatidylcholine: A Raman and infrared spectroscopic study. *Biophys. J.* **54**, 781–790.

WONG, P. T. T., and MANTSCH, H. H., (1988b). High-pressure infrared spectroscopic evidence of water binding sites in 1,2-diacyl phospholipids. *Chem. Phys. Lipids* **46**, 213–224.

WONG, P. T. T., and HUANG, C. (1989). Structural Aspects of pressure effects on infrared spectra of mixed-chain phosphatidylcholine assemblies in D_2O. *Biochemistry* **28**, 1259–1263.

WONG, P. T. T., GIRONS, I. S., GUILLOU, Y., COHEN, G. N., BARZU, O., and MANTSCH, H. H. (1989). Pressure-induced changes in the secondary structure of the Escherichia coli methionine repressor protein. *Biochim. Biophys. Acta.* **996**, 260–262.

WONG, P. T. T., and RIGAS, B. (1990). Infrared spectra of microtome sections of human colon tissues. *Appl. Spectrosc.* **44**, 1715–1718.

WONG, P. T. T., and ZAKIM, D. (1990). Structural and dynamic properties of a two-dimensional solution of hexadecane in aqueous lipid bilayers: A pressure-tuning infrared spectroscopic study. *J. Phys. Chem.* **94**, 5052–5056.

WONG, P. T. T. (1990a). Non-pressure-dependancy infrared absorption spectra recording, sample cell. U.S. Patent No. 4980551.

WONG, P. T. T. (1990b). Infrared spectra recording, high pressure sample holder. U.S. Patent No. 4970396.

WONG, P. T. T., PAPAVASSILIOU, E. D., and RIGAS, B. (1991). Phosphodiester stretching bands in the infrared spectra of human tissues and cultured cells. *Appli. Spectrosc.*, In press.

ZAKIM, D. and WONG, P. T. T. (1990). A high-pressure infrared spectroscopy study of the solvation of bilirubin in lipid bilayers. *Biochemistry* **29**, 2003–2007.