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Infrared Analysis of Bile

Analysis of gallstones by infrared absorption spectrum was established and this method was shown to be an effective and suitable means in the analyses of gallstones.[1~2] This work was further extended to the analysis of bile by infrared absorption spectra.

Bile contains bile acids, bile pigments, lipids, inorganic components, and many other substances, and detailed studies have been made on each of these components. The routine clinical examination of bile chiefly concerns the color, turbidity, and presence of bacteria, and analysis of chemical components is hardly carried out because the analysis requires complicated and difficult procedures.

There have never been any reports to date on the application of infrared absorption spectrum for analysis of bile and it was found from the present series of work that the infrared spectrum is also an excellent method for the analysis of bile, as was the case in that of gallstones.

1) Infrared Spectrum of Dried Whole Bile and its Interpretation :

Infrared absorption spectrum of dried whole bile gives informations on the components of bile, any changes thereof, and relative changes of the components. The infrared spectrum of the bile from the gallbladder shows approximately constant absorptions in healthy persons and, in spite of the bile being a mixture of numerous substances, a spectrum with many, fairly sharp absorptions can be obtained. The positions of main absorptions are at 796, 846, 852, 898, 913, 952, 982, 1001, 1017, 1052, 1079, 1111, 1171, 1201, 1229, 1311, 1405, 1465, 1551, 1655, and 1738 cm^{-1} and, although there are some shifts, these are overlapped absorptions of sodium glycocholate and taurocholate, cholesterol, lecithine, triglycerides, polysaccharides, and proteins. This fact was confirmed through separation of various bile components and comparison of absorptions of standard specimens. There are instances where absorption of sodium glycocholate is abnormally strong, bile acids are few in quantity, or cholesterol is abnormally great in quantity, and various changes in the components appear as the changes in spectral intensity.

1) G. Chihara, S. Yamamoto, H. Kameda : This Bulletin, **6**, 50(1958).
2) To be published.

The B bile and C bile collected by duodenal probe are fundamentally the same as that from gallbladder but the spectrum is somewhat different and further work is being continued. The difference in the spectra is natural from the difference in the components of liver bile and gallbladder bile but in this case, the spectrum is often interfered by the absorptions of inorganic salts. Removal of salts and other procedures are considered to be necessary for standardization.

In the case of a dog, infrared spectrum of its bile is far simpler than that in man and absorptions of taurocholic acid and lipids appear. The absorptions are almost constant and appear at 805, 856, 913, 952, 982, 1047, 1078, 1216, 1378, 1465, 1550, 1655, and 1740 cm^{-1}. The marked difference from the spectrum of human bile is the absence of strong absorption of sodium glycocholate at 1405 cm^{-1}. Variation in bile components after administration of ursodeoxycholic acid and other bile agents is now being followed. This kind of change mainly appears in the intensity of absorption of lipids at 1740 cm^{-1}. Infrared spectrum offers important means for studies in this field.

2) Extraction of Bile Lipids and their Infrared Spectra :

The bile was extracted with methanol and ether,[3] and infrared spectrum of the lipid so extracted was measured.[4~5] The absorptions appeared as the overlapping of those of lecithine, triglycerides, and cholesterol, mainly at 817, 869, 922, 971, 1063, 1089, 1171, 1235, 1374, and 1745 cm^{-1}, the absorption of lecithine being the strongest in intensity. Variation in the quantity of triglycerides can be observed from the ratio of absorptions of lecithine at 1071 and 1235 cm^{-1}, and that of triglycerides at 1171 cm^{-1}. The large quantity of cholesterol present can be judged from changes in spectral intensity of the absorptions at 1063 and 1089 cm^{-1}, and appearance of cholesterol absorptions at 769 and 835 cm^{-1}.

3) Analyses of Taurocholic Acid and Glycocholic Acid :

The above-mentioned method of extraction fails to extract bile acids which can be extracted by addition of hydrochloric acid to the residue left after extraction of lipids and extraction of the acid mixture with ether. The extracted human bile acid is a mixture of glycocholic acid and sodium taurocholate, and the spectrum appears as the overlapping of the absorptions of these substances. The relative ratio of these substances can be judged from the ratio of intensities of the absorption of a strong and broad S–O stretching frequency of taurocholic acid at 1216 cm^{-1} and that of C=O stretching absorption of glycocholic acid at around 1740 cm^{-1}. A more strict quantitative determination is now under study.

4) Ratio of Bile Acids and Total Lipid :

Addition of a small amount of methanol and hydrochloric acid to bile acid and extraction of this mixture with ether affords bile acid and total lipid. Both the bile acid and lipid are the mixture of several substances and their strict quantitative determination is difficult. Moreover, bile acid hardly dissolves in any of the solvents used for measurement of infrared spectrum, except in chloroform. Some informations can therefore be gained from intensity ratio of the ester absorption at around 1742 cm^{-1} and amide-I absorption of bile acid at 1660 cm^{-1}.

5) Proteins and Polysaccharides :

Addition of methanol or ethanol to bile acid generally produces a small amount of a precipitate whose infrared spectrum exhibits absorption of proteins or polysaccharides,

3) B. Borgstrom : Acta Physiol. Scand., **25**, 101(1952).
4) N. K. Freeman : Ann. N. Y. Acad. Sci., **69**, 131(1957).
5) G. Chihara, *et al.* : This Bulletin, **8**, No. 3(1960), to be published.

or a mixture of the two. In majority of cases, this is a mixture of polysaccharides and proteins and absorption of protein appears abnormally strong in the case of gallbladder disease.

Infrared spectral measurement of bile acid as a film on silver chloride does not give a good result due to predominant absorption of water.

The foregoing is a summary of observations made on approximately 600 infrared spectra obtained with bile and further work on them is being continued. It is hoped that a more detailed report will be made in the near future.

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