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127. Goro Chihara,*[1] Emiko Kobayashi,*[1] Ayako Mizushima,*[1] Kimiko Shirakuma,*[1] and Haruo Kameda*[2] : Medical and Biochemical Application of Infrared Absorption Spectra. IV.[1] Studies on Gall Stones by Infrared Spectra and their New Classification. (2).

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Earlier paper of this series[2] reported the application of infrared spectra for analysis of gall stones and this method was confirmed to be the most rapid and accurate for their analysis. The gall stones were found to be composed chiefly of cholesterol, calcium salt of bilirubin, and calcium carbonate.

Later, almost 800 infrared spectra of about 500 pieces of gall stone were examined and several kinds of new calculi were found, some containing calcium salt of fatty acids, a group of calculi chiefly containing calcium stearate, and those containing triglyceride and polysaccharides as the chief component. Analytical method for these calculi was also established and, together with the previously reported method, analysis of gall stones by infrared spectra may be said to be almost completely established.

Past classification of gall stones followed Aschoff's method[3] of structural observations with naked eyes and Anderson's method[4] by chemical composition, but neither are satisfactory in the light of results obtained in present series of work. Therefore, a new classification of gall stones, different from the present known ones, is herein proposed.

The result of these studies have revealed that there is a vast difference in the components of gall stones between urban and rural districts. In urban area, calculi with cholesterol as the chief component are found in large numbers whereas those with calcium salt of bilirubin as the chief component are found in rural area, there being very small incidence of cholesterol and increasing ratio of calculi containing calcium stearate. This is probably due to difference in dietary habits and is an interesting phenomenon.

Experimental

Apparatus—Infrared spectra were measured in the region of $2000\sim650\,cm^{-1}$ with Hilger H-800 infrared spectrophotometer provided with NaCl prism.

Material—A total of 474 pieces of gall stone taken out by surgical operation were used. These stones came from the following areas :

Metropolitan area : Tokyo University Hospital and other hospitals in Tokyo....290 pcs.
Medium cities : Hospitals in Nagasaki, Utsunomiya, and Kiryu ....125 〃
Rural area : Seihoku Hospital, Goshogawara, Aomori Pref...................... 58 〃

Besides the above, 70 pieces of gall stones taken out in autopsy by the Tokyo Medical Examiner Office were also used, making a grand total of 544 pieces of gall stone used as experimental material.

Method—Spectra were all measured in Nujol mull because this gave better result than the KBr disk method, differing from the case of urinary stones.[5]

Small calculi were measured as such but larger calculi were measured in over three places, such as the central portion, and inner and outer layers, so that the total number of spectra measured were almost 800. For one measurement, $1\sim3$ mg. of the sample was used.

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1) Part III. This Bulletin, 8, 222(1960).
2) G. Chihara, S. Yamamoto, H. Kameda : *Ibid.*, **6**, 50(1958).
3) C. Aschoff, A. Bacmeister : 'Die Cholelithiasis,' (1909). Fischer, Jena.
4) W. A. D. Anderson : 'Pathology,' 843(1953). Mosby Co., New York.
5) G. Chihara, N. Kurosawa, E. Takasaki : This Bulletin, **7**, 622(1959).

Results and Discussions

Examination of Classification of Gall Stones

Many methods have been used for classification of gall stones and many new classifications have been proposed with the development of test methods and increased clinical cases. Various improvements have been made on old classifications.

The most common is the classification of Aschoff (cf. A in Table I). This is a clinical and macroscopic classification, and is not necessarily appropriate. Other classifications used more commonly today are that of Bochus[6] from the point of gastroenterology (B in Table I) and that of Anderson[4] from chemical components (C in Table I), but none of these are satisfactory. Many of the calculi used as samples in the present work could not be classified by these past methods.

TABLE I. Past Classification of Gall Stones

A) Aschoff's Classification :
- 1) Radiate pure cholesterol stones
- 2) Combination stones
- 3) Cholesterol-calcium stones
  - (a) Layered cholesterol-calcium stones
  - (b) Cholesterol-pigment-calcium stones
- 4) Bilirubin stones
- 5) Choledochus stones
- 6) Rare stones

B) Bochus' Classification :
- 1) Radiate cholesterol stones
- 2) Pigment-calcium stones (pure pigment stones, mulberry stones)
- 3) Cholesterol-bilirubin calcium stones
- 4) Combination stones
- 5) Rare forms

C) Anderson's Classification :
- 1) Pure gallstones
  - (a) Crystalline cholesterol stone
  - (b) Bilirubin calcium stone
  - (c) Calcium carbonate stone
- 2) Mixed gallstones
  - (a) Cholesterol-bilirubin calcium stone
  - (b) Cholesterol-calcium carbonate stone
  - (c) Cholesterol-bilirubin calcium-calcium carbonate stone
- 3) Combined gallstones

A new classification of gall stones is now proposed based on chief components found by the series of infrared spectral analyses. It is possible to classify practically any kind of calculi clearly and accurately. Each group has not only different chemical and macroscopic characteristics but has significant clinical correlation[7] which gives important suggestions on the elucidation of causes for formation of gall stones, and for diagnosis and treatment of cholelithiasis. The new classification for gall stones is given in Table II. This classification is based on chief component of the calculi and such classification is possible because semi-quantitative consideration of components can be made through infrared absorption spectra.

Infrared Absorption Spectra

Infrared spectrum of a gall stone is presented as the overlapped spectra of its constituent components. In the earlier report,[2] analyses of gall stones containing cholesterol,

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6) H. L. Bochus : 'Gastroenterology,' III, 587(1946). W. B. Saunders, New York.
7) H. Kameda, S. Yamamoto, G. Chihara : Saishin Igaku, **13**, 445(1958).

Table II. New Classification of Gall Stones

1) Cholesterol as chief component :
   a) Pure cholesterol
   b) Mixture of cholesterol and calcium salt of bilirubin
   c) Mixture of cholesterol, calcium salt of bilirubin, and calcium carbonate
   d) Mixture of cholesterol and calcium carbonate
   e) Mixture of cholesterol and calcium phosphate
   f) Mixture of cholesterol, calcium carbonate, and calcium phosphate
2) Calcium salt of bilirubin as the chief component :
   a) Pure calcium salt of bilirubin
   b) Mixture of calcium salt of bilirubin and cholesterol
   c) Mixture of calcium salt of bilirubin, cholesterol, and calcium carbonate
   d) Mixture of calcium salt of bilirubin and calcium carbonate
   e) Mixture of calcium salt of bilirubin and calcium phosphate
3) Calcium carbonate as the chief component
4) Calcium stearate stone :
   a) Pure calcium stearate
   b) Mixture of calcium stearate and calcium salt of bilirubin
   c) Mixture of calcium stearate and cholesterol
   d) Mixture of calcium stearate, calcium salt of bilirubin, and cholesterol
   e) Mixture of calcium stearate and free stearic acid
5) Stones containing triglyceride
6) Stones containing polysaccharide
7) Protein stone
8) Slurried mixture of stones
9) Other stones

calcium salt of bilirubin, calcium carbonate, and proteins were described. The components newly found in gall stones from infrared spectra in the present series of work were stearic acid, calcium stearate, triglyceride, and polysaccharide. These fundamental components are combined in various ratios and various modes to make up the large number of gall stones listed in Table II. Main absorptions of these fundamental components are listed in Table III and those suited especially as key bands are marked with an asterisk.

Table III. Position of Infrared Absorption of the Chief Components in Gall Stones

Cholesterol : 716 (w), 728 (w), 795* (s), 836* (m), 926 (vw), 934 (vw), 952* (s), 982 (w), 1020 (s), 1052* (vs), 1080 (vw), 1108* (m), 1133 (m), 1168 (vw), 1192 (w), 1250 (vw), 1275(vw)1330 (w).
Calcium salt of bilirubin : 750 (w), 866 (w), 912 (w), 932 (w), 948 (w), 988 (m), 1051 (m), 1104 (w), 1186 (w), 1217 (w), 1250* (s), 1568* (vs), 1627*(vs), 1662* (vs), 1702* (vs).
Calcium stearate : 713* (s), 1109* (m), 1300 (w), 1538* (vs), 1576* (vs).
Stearic acid : 718* (s), 950* (s), 1702* (vs).
Triglyceride : 712 (m), 1172* (s), 1739* (vs).
Calcium carbonate (calcite) : 1410~1450* (vs, broad), 874* (s), 712 (w).
Calcium crabonate (aragonite) : 700 (w), 712 (w), 860* (s), 1420~1480* (vs, broad).
Calcium phosphate : 960 (w), 1000~1100* (vs).
Polysaccharide : 1000~1100* (vs, broad), 1700* (s).
Protein : ca. 1550* (s), 1640~1660* (s).
m=medium, s=strong, v=very, w=weak. * Key band of the component.

Some of the infrared spectra of gall stones measured newly in the present series of work are indicated in Figs. 1~6.

1) Fig. 1 is the spectrum of pure calcium stearate stone and the spectrum agrees completely with the absorption of synthesized pure calcium stearate. The two strong absorptions at 1538 and 1576 cm^{-1}, and absorptions at 713 and 1109 cm^{-1} are the characteristic bands.

2) There were some calculi in which calcium stearate and free stearic acid were mixed. The spectrum is an overlapped absorptions of stearic acid on that of calcium stearate.

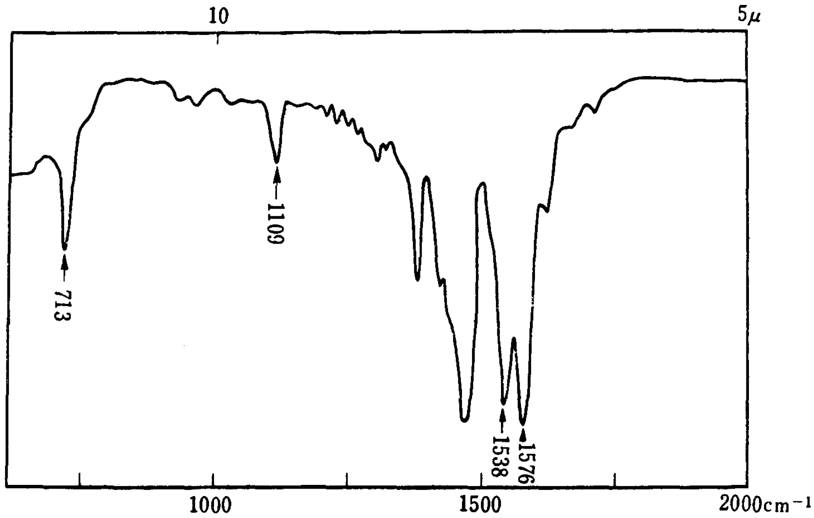

Fig. 1. (a) Calcium Stearate Stone

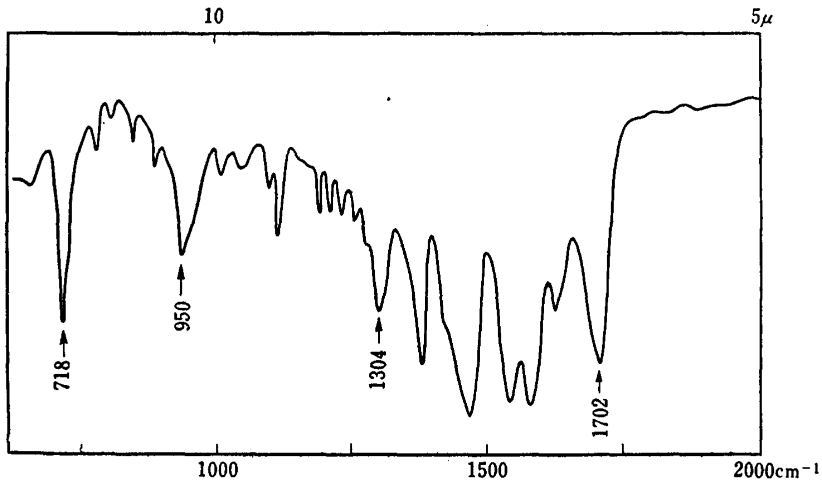

Fig. 1. (b) Calcium Stearate-Stearic Acid Stone

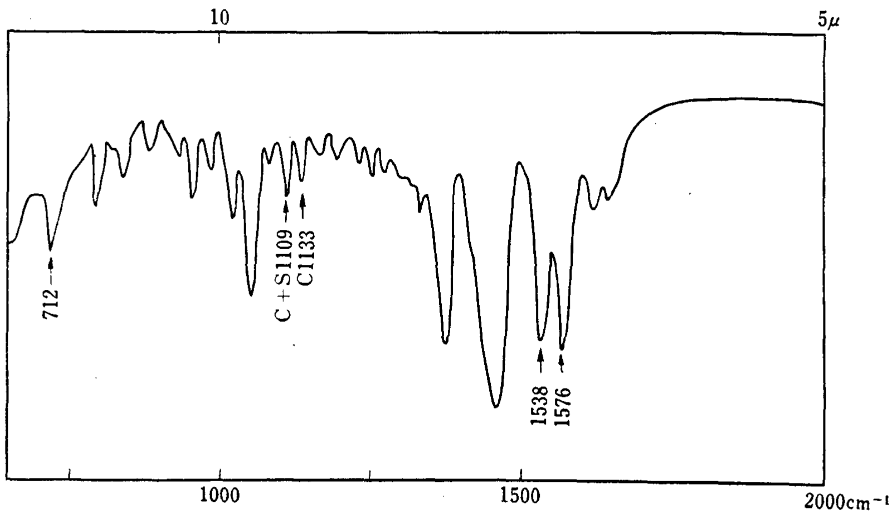

Fig. 2. Cholesterol (C)-Calcium Stearate (S) Stone

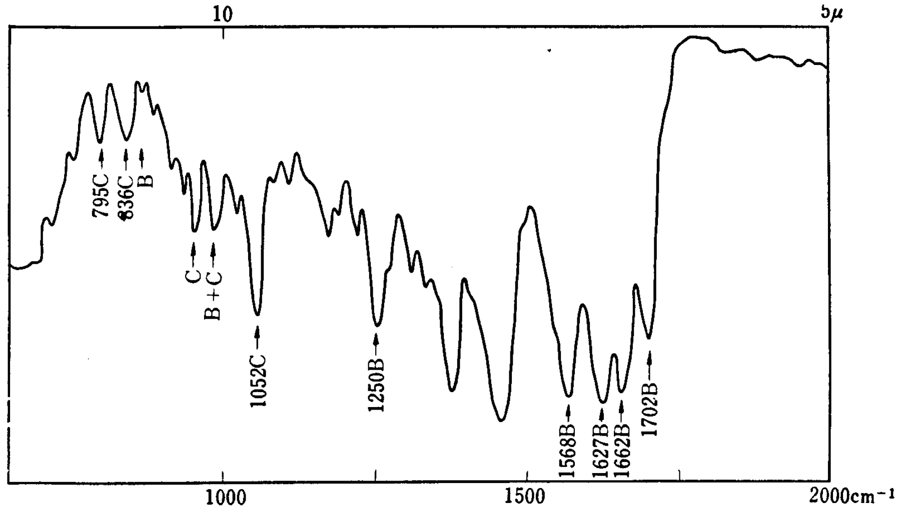

Fig. 3. Cholesterol (C)-Bilirubin Calcium (B) Stone

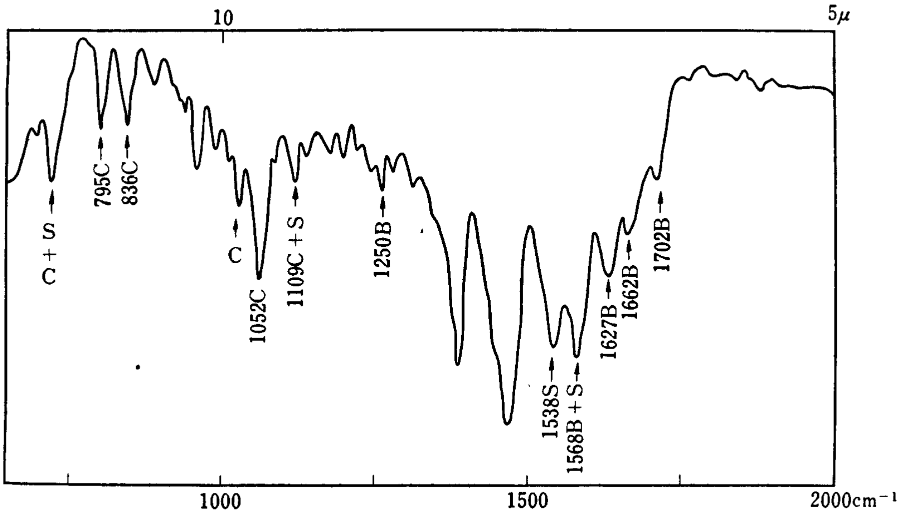

Fig. 4. Cholesterol-Bilirubin Calcium-Calcium Stearate Stone

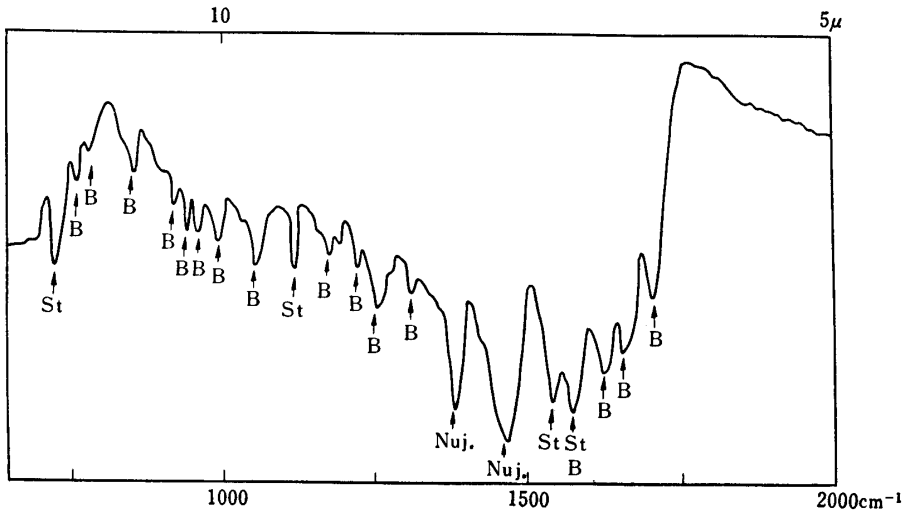

Fig. 5. Calcium Stearate-Bilirubin Calcium Stone

The characteristics of this spectrum are the very strong carboxyl absorption at 1702 cm^{-1} and strong absorptions at 718, 950, and 1304 cm^{-1}. There have been incidence of calcium stearate stone but those containing free acid had been unknown.

3) Calculi containing cholesterol and calcium stearate were found. Characteristic bands are the two strong absorptions at 1538 and 1576 cm^{-1}. Two very weak absorptions are often found in these positions in cholesterol stone and this may be considered to indicate the presence of a trace of calcium stearate. On the other hand, there often appears one weak absorption at 1550 cm^{-1} and the absorption of cholesterol at 1052 cm^{-1} sometimes becomes broad. This is considered to be absorption of cholic acid bound with amino acid. With increasing quantity of calcium stearate in the calculi containing cholesterol and calcium stearate, the absorption at 712 cm^{-1} becomes gradually stronger and, inversely, the absorption of cholesterol at 1109 cm^{-1} becomes stronger than that of 1133 cm^{-1}.

4) Fig. 3 is the infrared spectrum of calculus containing cholesterol and calcium salt of bilirubin as the chief component. This spectrum has been included because the spectrum of a similar stone published earlier*[3] was found to have been taken with a sample containing a small amount of calcium stearate. The absorption at 1627 cm^{-1} is stronger than that at 1568 cm^{-1}. When a small amount of calcium stearate is present, the absorption at 1568 cm^{-1} becomes stronger than that at 1627 cm^{-1} and an absorption appears at 1538 cm^{-1}.

Fig. 4 is the spectrum of a typical calculus containing a mixture of cholesterol, calcium salt of bilirubin, and calcium stearate in which the quantity of calcium stearate is very large. Fig. 5 is the spectrum of a calculus containing calcium stearate and calcium salt of bilirubin.

5) Fig. 6 is the spectrum of a calculus containing triglyceride. The characteristic bands are the extremely strong absorption at 1739 cm^{-1} and a strong, somewhat broad absorption at 1172 cm^{-1}. The absorptions indicated with F in Fig. 6 are those of triglyceride and this is a new kind of calculus. Triglyceride stones also containing calcium salt of bilirubin and others have also been found.

6) Spectrum of stones containing tricalcium diphosphate was shown in an earlier work.[2] Stones containing polysaccharide have also been found. Spectrum of these stones shows a very strong and broad absorption at 1000～1100 cm^{-1}, same as in calcium phosphate stone. Distinction between the two are the presence or absence of a strong absorption

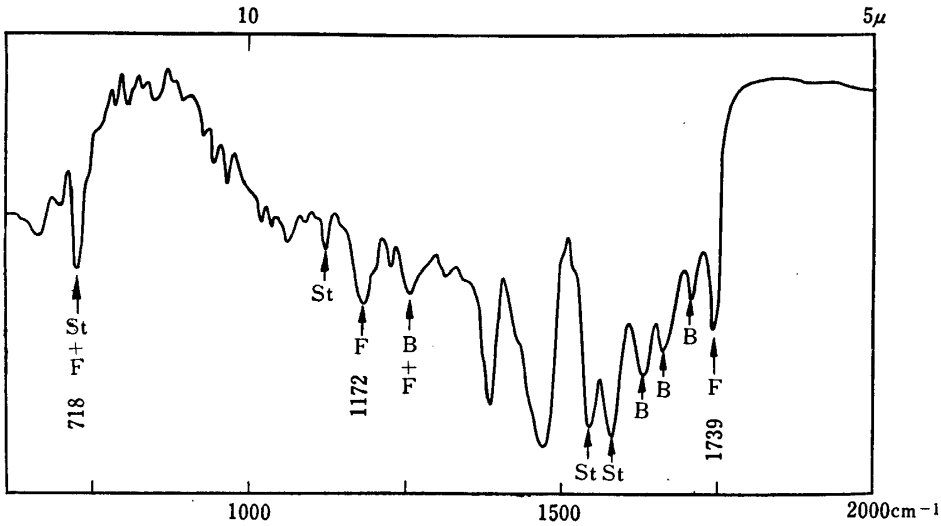

Fig. 6. Triglyceride (F)-Bilirubin Calcium-Calcium Stearate (St) Stone

*3 Fig. 3 on p. 51 of the literature cited in Reference (2).

of polysaccharide at around 1700 cm^{-1} and the presence or absence of ash after incineration.
7) By analysis of approximately 500 pieces of gall stones by infrared spectrum, practically all the stones were classified into one of the categories listed in Table II. The slurried mixed stone in that Table is a mass of necrotic tissue and the other stones include several kinds of new calculi. Infrared spectrum of one of them suggested a substance of cholic acid series and examinations are now being continued.

Difference in Gall Stone Components between Urban and Rural Areas

Clinical application of results obtained by such infrared spectral analysis of calculi has been described elsewhere.[7] Consideration of these results by geographic districts showed that there is a marked difference in components according to whether the stone came from large metropolitan area, smaller cities, or rural districts. Classification of chief components according to areas is shown in Table IV.

TABLE IV. Classification of Gall Stones by Chief Components and Difference between Urban and Rural Areas

Chief component	Incidence (no.) and percentage (%)		
	Metropolitan area	Medium cities	Rural area
Cholesterol	207 (67.9)	51 (40.8)	4 (6.8)
Bilirubin Ca	66 (22.7)	65 (52.0)	44 (74.6)
Ca stearate	6 (2.1)	5 (4.0)	9 (15.3)
Ca carbonate	6 (2.1)	1 (0.8)	0
Triglyceride	2 (0.7)	1 (0.8)	1 (1.7)
Polysaccharide	1 (0.3)	2 (1.6)	1 (1.7)
Protein	2 (0.7)	0	0
Total	290	125	59

As will be clear from this table, cholesterol is overwhelmingly large but calcium salt of bilirubin is rare in the metropolitan area. On the other hand, this calcium salt of bilirubin is overwhelmingly large in rural villages and cholesterol is extremely rare, with increased appearance of calcium stearate in its stead. This fact is assumed to be due to the difference in dietary habits of the Japanese, larger quantity of meat being taken by people living in large cities and carbohydrates as staple food in rural villages. This difference in the correlation between cholesterol and stearate should be noted.

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Summary

Infrared absorption spectra of over 500 pieces of gall stone were measured and analysis of gall stones by infrared spectra was established. This is the best method now known for the analyses of gall stones. Spectra were newly obtained for a group of calculi containing stearic acid, calcium stearate, triglyceride, and polysaccharide. Almost all of the gall stones are composed of a combination of such substances and cholesterol, calcium salt of bilirubin, calcium carbonate, calcium phosphate, and proteins, in various ratios and in variety of modes.

A new classification has been proposed based on these chief components. It was also found that there is a vast difference in gall-stone components between urban and rural areas. Cholesterol, which is found in metropolitan area, is rare in rural villages and in its stead ratio of calcium stearate to calcium salt of bilirubin increases.

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