To explore reproductive risk factors for asymptomatic gallstones in females, we conducted a nested case-control study. The study comprised 126 cases (women with asymptomatic gallstones) and 378 controls (women free from gallstones and other diseases of the liver and biliary tract) among 3,927 women aged 40 years or more in Y town, Hokkaido, Japan. Controls were randomly selected with an allocation ratio of 1:3 from 3,599 cohort women, matching to cases for sex (female), age (±2 years), residential area, and year of examination. Odds ratio analysis revealed the following major findings. (1) Women married for the first time at 22 years old or later are likely to be at greater risk of asymptomatic gallstones with odds ratios of 1.60 (95% confidence interval: 0.97-2.64); 1.68 (0.96-2.94) for postmenopausal women. (2) First full-term delivery at 23 years old or later tended to increase the risk by 1.63 (0.97-2.77); 1.63 (0.90-2.9) when postmenopausal. (3) Pregnancy of 5 times or more also appeared to elevate the risk by 1.47 (0.97-2.22); 1.55 (0.94-2.57) when postmenopausal. Two odds ratios obtained by logistic regression analysis are significant: 1.67 (1.14-3.32) and 1.59 (1.01-2.52) for age at first marriage and total number of pregnancy, respectively. Finally, we emphasize that this is the first epidemiological investigation in Japan which explored an association between reproductive episodes and asymptomatic gallstone formation in women. J Epidemiol, 1993; 3: 91-97.

asymptomatic gallstones, females, reproductive episodes, nested case-control study
manifesting gallstones as a clinical disease.

To our knowledge, this is the first epidemiological study in Japan, in which reproductive factors were investigated in relation to asymptomatic gallstones in women.

MATERIALS AND METHODS

Population at risk, screening for gallstones, and collection of relevant data

This study was conducted at Y town, which is inhabited by approximately 20,000 population and located in the southern part of Hokkaido Island, with major industrial makeup of agricultural and dairy farming and fishery including scallop cultivation.

In the period from 1983 to 1989, 3,927 women aged 40 years or more were examined by ultrasonography using a Aloka SSD-256, realtime, linear-array scanner with a 3.5-MHz probe (Aloka Co., Ltd., Japan) under fasting condition. Such information were simultaneously collected or measured as date of birth, residence, height, weight, skinfold, urinalysis, electrocardiogram, eye fundus photograph, and chest x-ray examination. Biochemical examination was conducted for blood collected by venopuncture under fasting condition. To obtain personal information on past history, lifestyle habits, dietary habits, and other epidemiological items, a self-administered questionnaire was used, which was distributed about one month before examination and reviewed at examination by five public health nurses who were specifically trained for this study. When answers were missing, vague or contradictory, collection or correction was made by their direct interview. The review of each questionnaire by nurses was performed irrelevantly of the findings obtained by abdominal scanning. These data collection and examination were annually conducted every summer since 1983.

Among various information thus collected, we can only use some of them in this particular study, because study design is a nested case-control study, in which information to be discussed should be restricted to those that existed sometime before the detection of asymptomatic gallstones. Reproductive episodes to be discussed in this paper are representatively such kind of information. Obesity is regarded as one of relevant factors to symptomatic gallstones mostly in cross-sectional analysis. Past episode of obesity was, however, not available in our data collection, and, therefore, it was not included as a variable in the analysis.

Case identification and eligibility

Among a total of 3,927 women examined, we identified 201 women with gallstones and/or past history of cholecystectomy. Among 201 women, those underwent cholecystectomy were 63 women, followed by 7 stone formers not cholecystectomized, but experienced three typical symptoms of biliary colic, jaundice and fever in the past years, and 5 stone formers who had no symptoms at detection, but later developed above three symptoms in at least two years after detection of gallstones. Excluding these 75 women, as a consequence, we could finally identified 126 asymptomatic gallstone formers, who are defined as cases in this study.

Control selection

We derived a source of controls from 3,927 women by excluding 201 stone formers and 127 women who demonstrated abnormal findings in the liver and biliary tract at examination. From 3,599 women, we randomly selected three controls per case by a pair matching procedure. Matching variables are sex (females), age (±2 years), residential area, and year of examination (all examinations were conducted in summer every year). Finally we could successfully selected 378 women, who could satisfy above inclusion and exclusion criteria and who were also proved to be free from gallstones either symptomatic or asymptomatic for at least two years after examination.

Data analysis

Data on 126 cases and 378 controls were analyzed using matched-set odds ratio with 95% confidence interval and Mantel-Haenszel chi-square statistics to assess the significance of differences using two-sided test of significance. For multivariate analysis, we used a conditional logistic regression model. To examine the relationship between the risk of asymptomatic gallstone and reproductive episodes by menopausal status, cases and controls were divided into women still menstruating (premenopausal) and women who had stopped menstruating naturally or artificially (postmenopausal). In this analysis, we calculated age-adjusted odds ratio with 95% confidence interval by Mantel-Haenszel Extension Method and used an unconditional logistic regression model. Data analysis was all processed at Nagoya University Computation Center and Aichi Medical University Information Processing Center, using SAS and related programs.

RESULTS

Table 1 shows the age distribution of our study subjects. Mean ages of the subjects were insignificantly different.
Table 2 presents an association of asymptomatic gallstones with selected background variables such as educational attainment, physical exercise, smoking and drinking habits, and past history of diseases. These variables were not linked at all with a risk of asymptomatic gallstone formation.

Table 3 gives an association with reproductive episodes. Each reference category, to which odds ratio is set to be 1.00, was decided such that it contains about one thirds or two thirds of controls. For example, when dividing by age at menarche, a cutoff point of 15 years old could give us 123 controls (33.1%) in the reference category. Age at menarche was associated with neither increased nor decreased risk of asymptomatic gallstones. Mean ages and standard deviations at menarche were 14.8±1.7 years for cases, and 15.0±3.0 for controls; not differing significantly.

Out of 126 cases and 378 controls, 34 and 90 are premenopausal, respectively. Mean ages at menopause calculated by excluding them were 48.9±4.2 years for cases, and 47.9±5.3 for controls; being insignificantly different. Odds ratio analysis revealed a ratio of 1.06 (95% confidence interval (CI): 0.65-1.72) for those who became menopausal at age less than 48 years. Total years of menstruation, defined as the period in years between age at menarche and age at menopause or at moment, were on an average 33.9±4.4 years for cases and 32.7±5.2 for controls, without significant difference.

Women married for the first time at 22 years old or later and those with first full-term delivery at 23 years old or later were likely to be at greater risk of asymptomatic gallstones; magnitudes of an increased risk being 1.60 (95% CI: 0.97-2.64) and 1.63 (0.97-2.77), respectively. Mean ages at first marriage and at first full-term delivery were 23.4±2.7 years and 24.6±3.5 in cases, respectively, and 23.0±2.9 years and 24.1±3.9 in controls, correspondingly. Differences in these mean ages were not significant.

Total number of pregnancy was also likely to be associated, but that of live birth was not. Being pregnant 5 times or more demonstrated an increased risk of 1.47 (95% CI: 0.97-2.22). Mean numbers of pregnancy and live birth were 4.3±2.0 times and 3.4±1.8 in cases, respectively, and 4.0±1.9 times and 3.3±1.8 in controls, correspondingly; these being not significantly different.

Table 1. Age distribution of study subjects (at entry).

| Age (years) | Cases | Controls |
|------------|-------|----------|
| 40-49      | 27 (21.4) | 80 (21.2) |
| 50-59      | 43 (34.1) | 119 (31.5) |
| 60-69      | 50 (39.7) | 163 (43.1) |
| 70-        | 6 (4.8) | 16 (4.2) |
| Total      | 126 (100.0) | 378 (100.0) |

| Mean Age (± S.D.) | 57.4 (± 8.49) | 57.5 (± 8.36) |

Table 2. Odds ratios and 95% confidence intervals for asymptomatic gallstones by selected lifestyle habits and past history of diseases.

| factors | Cases N=126 (%) | Control N=378 (%) | Odds ratio | 95% Confidence interval | P-value |
|---------|----------------|------------------|-------------|-------------------------|---------|
| Education (years) | | | | | |
| <18 | 102 (81.0) | 300 (79.4) | 1.00 | | |
| ≥18 | 24 (19.0) | 78 (20.6) | 0.90 | 0.52-1.59 | 0.73 |
| Exercise (after 20 years old) | | | | | |
| Yes | 122 (96.8) | 361 (95.5) | 1.00 | | |
| No | 4 (3.2) | 17 (4.5) | 0.70 | 0.18-2.22 | 0.48 |
| Cigarette smoking (Former and current) | | | | | |
| No | 118 (93.7) | 351 (92.9) | 1.00 | | |
| Yes | 8 (6.3) | 27 (7.1) | 0.88 | 0.22-2.63 | 0.83 |
| Alcohol use | | | | | |
| No | 109 (86.5) | 338 (89.4) | 1.00 | | |
| Yes | 17 (13.5) | 40 (10.6) | 1.32 | 0.68-2.78 | 0.42 |
| Past history of illness | | | | | |
| Gastro-duodenal ulcer | | | | | |
| No | 108 (85.7) | 340 (89.9) | 1.00 | | |
| Yes | 18 (14.3) | 38 (10.1) | 1.49 | 0.75-2.33 | 0.24 |
| Appendicitis (Appendectomy) | | | | | |
| No | 97 (77.0) | 261 (69.0) | 1.00 | | |
| Yes | 29 (23.0) | 117 (31.0) | 0.67 | 0.41-1.09 | 0.11 |
| Diabetes | | | | | |
| No | 121 (96.0) | 364 (96.3) | 1.00 | | |
| Yes | 5 (4.0) | 14 (3.7) | 1.07 | 0.34-3.57 | 0.88 |
| Coronary heart disease | | | | | |
| No | 110 (87.3) | 346 (91.5) | 1.00 | | |
| Yes | 16 (12.7) | 32 (8.6) | 1.57 | 0.72-3.13 | 0.25 |
Table 3. Odds ratios and 95% confidence intervals for asymptomatic gallstones by reproductive episode.

| factors                          | Cases N=126. (%) | Controls N=378. (%) | Odds ratio | 95% Confidence interval | P-value |
|---------------------------------|------------------|---------------------|------------|-------------------------|---------|
| Age at menarche (years)         | ≥15 40 (31.8)    | 123 (33.1)          | 1.00       |                         |         |
|                                 | <15 86 (68.3)    | 249 (66.9)          | 1.06       | 0.69-1.64               | 0.79    |
| Age at first marriage (years)   | <22 24 (19.4)    | 101 (27.8)          | 1.00       |                         |         |
|                                 | ≥22 100 (80.7)   | 263 (72.3)          | 1.60       | 0.97-2.64               | 0.06    |
| Age at first full-term delivery (years) | <23 21 (17.2) | 87 (25.4)          | 1.00       |                         |         |
|                                 | ≥23 101 (82.8)   | 256 (74.6)          | 1.63       | 0.97-2.77               | 0.07    |
| Numbers of pregnancy (times)    | <5 65 (52.9)     | 219 (62.2)          | 1.00       |                         |         |
|                                 | ≥5 58 (47.2)     | 133 (37.8)          | 1.47       | 0.97-2.22               | 0.07    |
| Numbers of live birth (times)   | <4 75 (61.5)     | 209 (60.2)          | 1.00       |                         |         |
|                                 | ≥4 47 (38.5)     | 138 (39.8)          | 0.95       | 0.62-1.45               | 0.81    |

Table 4 summarizes age-adjusted odds ratio with 95% CI obtained from separate examinations by menopausal status.

In premenopausal women, pregnancy of 5 times or more alone demonstrated an odds ratio of a little less than two without any statistical significance. In postmenopausal women, age at first marriage of 22 years or later, age at first full-term delivery of 23 years or later, and pregnancy of 5 times or more appeared to increase the risk of asymptomatic gallstones, with age-adjusted odds ratio of 1.68 (0.96-2.94), 1.63 (0.90-2.95), and 1.55 (0.94-2.57), correspondingly. No material effects were, however, noted by age at menarche and total number of live birth in pre- and post-menopausal women, and by ages at first marriage and at first full-term delivery in premenopausal women.

We further analyzed a whole data set by a conditional logistic regression model and two menopausal data sets by an unconditional logistic regression model, including all reproductive episodes: ages at menarche, menopause, first marriage and first full-term delivery, and total numbers of pregnancy and live birth. Three logistic regression analyses yielded only one significant result, as summarized in Table 5. Separate multivariate analysis by menopausal status did not detect any significant reproductive episodes which either increase or decrease the risk of asymptomatic gallstone formation. Significant odds ratios, detected by multivariate analysis on a whole data set, were 1.67 (95% CI: 1.14-3.32) for age at first marriage of 22 years or later and 1.59 (95% CI: 1.01-2.52) for pregnancy of 5 times or more.

**DISCUSSION**

Gallstones have usually been investigated among symptomatic patients or those already experienced cholecystectomy, but rarely on those with asymptomatic stones. In Japan, in particular, few studies have been undertaken by contrasting those with and those without asymptomatic gallstones, which was, therefore, a main design of the present study.

We could clarify two major reproductive risk factors in our study, which significantly increase the probability of gallstone formation: first marriage at 22 years old or later and pregnancy of 5 times or more.

To our knowledge, age at first marriage has hardly ever been assessed in relating with either symptomatic or asymptomatic gallstones. Two studies, however, examined age at first birth, which may be in generally parallel with age at first marriage, in association with symptomatic gallstones. One study reported that the risk of symptomatic gallstones was about two-fold when age at first birth was 31 years or more, suggesting an association between older age at first birth and symptomatic gallstones. Another study, a case-control study, obtained an opposite finding that average age at first birth was significantly lower in patients with symptomatic gallstones (21.8 years old) than in controls (23.1 years old) among women aged less than 50 years, but average age at first birth was not significantly different at all between cases and controls when they aged 50 years or older.

An association of parity or frequency of pregnancy has been relatively often examined with symptomatic gallstones, but rarely with asymptomatic gallstones. According to an investigation, which examined 612 middle-aged women with symptomatic gallstones, no significant risk differential was detected between nulliparous and parous women. And also no elevation of risk for symptomatic gallstones was found by parity among 632 female symptomatic patients aged 40-69 years. In contrast to these investigators, frequent pregnancy or parity was reported as a significant
| factors                          | Pre-menopause (Mean age = 46.9 ± 7.6) | Post-menopause (Mean age = 60.2 ± 6.2) |
|---------------------------------|-------------------------------------|---------------------------------------|
|                                 | Cases N = 34. (%) | Controls N = 90. (%) | Odds ratio (95% C.I.) | P-value | Cases N = 92. (%) | Controls N = 288. (%) | Odds ratio (95% C.I.) | P-value |
| Age at menarche (years)         | ≥15 7 (30.6) | 14 (16.1) | 1.00 | 0.56 | 33 (35.9) | 109 (38.3) | 1.00 | 0.67-1.77 | 0.72 |
|                                 | <15 27 (79.4) | 73 (83.9) | 0.73 (0.26-2.11) | 0.56 | 59 (64.1) | 176 (61.8) | 1.09 (0.67-1.77) | 0.72 |
| Age at first marriage (years)   | <22 5 (15.2) | 15 (16.7) | 1.00 | 0.93 | 19 (20.9) | 86 (31.4) | 1.00 | 0.96-2.94 | 0.07 |
|                                 | ≥22 28 (84.9) | 75 (83.3) | 1.05 (0.34-3.24) | 0.93 | 72 (79.1) | 188 (68.6) | 1.68 (0.96-2.94) | 0.07 |
| Age at first full-term delivery (years) | <23 4 (11.8) | 14 (15.9) | 1.00 | 0.56 | 17 (19.3) | 73 (28.6) | 1.00 | 0.93-3.03 | 0.09 |
|                                 | ≥23 30 (88.2) | 74 (84.1) | 1.42 (0.43-4.66) | 0.56 | 71 (80.7) | 182 (71.4) | 1.66 (0.93-3.03) | 0.09 |
| Numbers of pregnancy (times)   | <5 23 (67.7) | 72 (80.0) | 1.00 | 0.18 | 42 (47.2) | 147 (56.1) | 1.00 | 0.94-2.57 | 0.09 |
|                                 | ≥5 11 (32.4) | 18 (20.0) | 1.89 (0.75-4.75) | 0.18 | 47 (52.8) | 115 (43.9) | 1.55 (0.94-2.57) | 0.09 |
| Numbers of live birth (times)  | <4 30 (88.2) | 76 (84.4) | 1.00 | 0.30 | 45 (51.1) | 133 (51.8) | 1.00 | 0.69-1.92 | 0.65 |
|                                 | ≥4 4 (11.8) | 14 (15.6) | 0.45 (0.10-2.04) | 0.30 | 43 (48.9) | 124 (48.3) | 1.13 (0.69-1.92) | 0.65 |
Table 5. Odds ratios and 95% confidence intervals, obtained by conditional logistic regression analysis on whole data-set.

| Risk Factor | Beta | Standard Error | Chi-square | Odds Ratio | 95% Confidence Interval |
|-------------|------|----------------|------------|------------|-------------------------|
| Age at first marriage (years) | 0.67  | 0.27           | 5.95       | 1.67       | (1.14-3.32)             |
| Numbers of pregnancy (times) | 0.47  | 0.23           | 3.96       | 1.59       | (1.01-2.52)             |

a Model chi-square = 4.52 (P = 0.0336) R = 0.089, 0 ≤ 22, 1 ≥ 22
b Model chi-square = 8.46 (P = 0.0146) R = 0.117, 0 ≤ 5, 1 ≥ 5

Included in the model are age at menarche, menopause, first marriage and first full-term delivery, and numbers of pregnancy and live birth.

Risk factor incriminated for symptomatic gallstones or cholecystectomy, though not for asymptomatic gallstones.

Effects of pregnancy and menstrual cycle are reported in relation to gallstone formation as follows: (1) both fasting and residual volumes of the gallbladder after meal are increased in pregnancy, (2) during the luteal phase of the menstrual cycle, gallbladder emptying is impaired, (3) bile acid metabolism is altered by pregnancy, (4) choledolithiasis is experimentally demonstrated by prolonged progesterone treatment in rabbits, and (5) contractile properties of the gallbladder are impaired in guinea pig in vitro in the presence of progesterone.

Transient obesity at pregnancy may also be related to gall-stone formation. Becoming obese gradually with advancing months of pregnancy might lead to poor contracting of the gallbladder due to poor elevation of the diaphragm, which finally results in development of biliary stasis. Biliary stasis is considered as a biochemical basis of favouring cholesterol gallstone formation by secreting cholesterol-saturated bile, nucleation of cholesterol crystals and growth of stones in the gallbladder.

We conducted this study at Y town, inhabitants of which have never been screened for gallstones by ultrasonography. It means that women found to have asymptomatic gallstones are all detected for the first time and those who have not sought for any medical attention or treatment. Referent group was selected from 3,599 women without any abnormalities in the liver and biliary tract and gallstones proven by ultrasonography, with a ratio of 1:3 by individually matching to cases for sex, age, residence and year of examination. Besides this matching procedure, we traced the study subjects for at least two years, and confirmed that all cases remained as asymptomatic and controls as non-stone formers. This implies further that the two groups are reasonably comparable. Selection bias of the study subjects is also not applicable, since they are not subjectively selected by any means, and all eligible cases are included, and all controls are randomly selected.

Information on reproductive episodes collected by a self-administered questionnaire are reviewed and corrected by direct interview by five public health nurses. They are also inhabitants in Y town and have close contacts with examinees. This indicates that examinees are not likely to answer incorrectly to private questions such as reproductive episodes. Besides this unlikeliness, all information are collected without knowing whether an interviewee will be included as a study subject or not, since the present study is later constructed as a nested case-control study. Despite these reliabilities, possibility of misclassification bias particularly by menopausal status can not be excluded, because whether postmenopausal or not is solely dependent upon the answer of each woman. Inter- and intra-interviewer variations, however, are believed to be minimal, since all interviewers were trained beforehand by standardizing whole interview procedures.

Finally, we could identify the two major reproductive risk factors for asymptomatic gallstones in females, though their medical implications in gallstone formation are not yet fully understood. It should, however, be particularly emphasized that the present findings are obtained for asymptomatic gallstones, but not for symptomatic gallstones. Epidemiologic risk factors for gallstone formation are believed to be not only these reproductive factors but also various life-style factors not readily assessed in the present nested case-control study. And therefore other types of epidemiological investigations should be undertaken to evaluate an association of diets, body weight changes, or serum levels of total cholesterol and triglyceride.

REFERENCES
1. Scragg RKR, McMichael AJ, Baghurst PA. Diet, alcohol, and relative weight in gallstone disease: a case-control study. Br Med J. 1984; 288: 1113-1119.
2. Friedman GD, Kannel WB, Dawber TR. The
epidemiology of gallbladder disease: observations in the Framingham study. J Chron Dis, 1966; 19: 273-292.

3. Bernstein RA, Giefer EE, Vieira JJ, Werner LH, Rimm AA. Gallbladder disease -II Utilization of the life table method in obtaining clinically useful information. J Chron Dis, 1977; 30: 529-541.

4. Hanis CL, Ferrell RE, Tuulio BR, Schull WJ. Gallbladder disease epidemiology in Mexican Americans in Starr county, Texas. Am J Epidemiol, 1985; 122: 820-829.

5. Thijs C, Knipschild P, Engelshoven JP. The prevalence of gallstone disease in Dutch population. Scand J Gastroenterol, 1990; 25: 155-160.

6. Meltzer AL, Heymsfield S, Grundy SM. The lithogenic index - A numerical expression for the lithogenicity of bile. Gastroenterology, 1972; 62: 499-501.

7. Nestel PJ, Schreiman PH, Ahrens Jr EH. Cholesterol metabolism in human obesity. J Clin Invest, 1973; 52: 2389-2397.

8. Rome Group for the Epidemiology and Prevention of Cholelithiasis (GRPCO) Prevalence of gallstone disease in an Italian adult female population. Am J Epidemiol, 1984; 119: 796-805.

9. Beral V. Long term effects of childbearing on health. J Epidemiol Community Health, 1985; 39: 343-346.

10. Sherlock S. Diseases of the Liver and Biliary System 3rd edit. F.A. Davis, Philadelphia, 1963.

11. Gilat T, Feldman C, Halpern Z, Dan M, Bar-Meir S. A study of a possible association between breast cancer and gallbladder disease. Am J Epidemiol, 1990; 131: 836-844.

12. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Nat Cancer Inst, 1959; 22: 719-749.

13. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic Research, Principles and Quantitative Methods. Van Nostrand Reinhold Company, New York, 1982.

14. Mantel N. Chi-square tests with one degree of freedom - Extensions of the Mantel-Haenszel procedure. Am Stat Assoc J, 1963: 58: 690-700.

15. Breslow NE, Day NE. Statistical Methods in Cancer Research Volume I—the Analysis of Case-Control Studies. IARC Scientific Publications, No.33. Lyon, 1980.

16. Pixley F, Wilson D, McPherson K, Mann J. Effect of vegetarianism on development of gall stones in women. Br Med J, 1984; 288: 1795-1799.

17. Strom BL, Tamragouri RN, Morse ML, et al. Oral contraceptives and endogenous oestrogen in gallstone disease—case-control study. Br Med J, 1986; 291: 719-724.

18. Dyer A, Rosenthal M, Hazuda HP, Comeaux PJ, Stern MP. Socioeconomic status and the prevalence of clinical gallbladder disease. J Chron Dis, 1985; 38: 1019-1026.

19. Ranelletti FO, Piantelli M, Farinon AM, Zanella E, Capelli A. Estrogen and progesterone receptors in the gallbladders from patients with gallstones. Hepatology, 1991; 14: 608-612.

20. Everson JT, McKinley C, Lawson M, Johnson M, Kern F. Gallbladder function in the human female: effect of the ovulatory cycle, pregnancy and contraceptive steroids. Gastroenterology, 1992; 102: 711-719.

21. Braun B, Dormeyer HH. Changes in gallbladder motor function during the female cycle: a risk factor leading to gallstone formation? Klin Wochenschr, 1982; 60: 1357-1362.

22. Kern F Jr, Everson GT, DeMark B, et al. Biliary lipids, bile acids, and gallbladder function in the human female—effects of pregnancy and the ovulatory cycle. J Clin Invest, 1981; 68: 1229-1242.

23. Imamoglu K, Wangensteen SL, Root HD. Production of gallstones by prolonged adadministration of progesterone and estradiol in rabbits. Surg Forum, 1959; 1: 246-249.

24. Ryan JP, Pellechcia D. Effect of progesterone pretreatment on guinea pig gallbladder mortality in vitro. Gastroenterology, 1982; 83: 81-83.

25. Okuda T, Mitamura K, Miyagawa S, et al. A Scanning Electron Microscopic Study of Gallstone Development in Man. Labo Invest, 1974; 31: 696-704.