1297 Physiological and Pharmacological Regulation of Potassium Channels of Human Colonic Epithelium

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We previously characterised Na⁺, K⁺, Cl⁻, channels; a Cl⁻-HCO₃-anion exchange; a Na⁺-K⁺-Cl⁻ co-transporter and a Na⁺-K⁺-ions pump in the sodium absorptive cells of the human colonic epithelium. In this study we examined regulation of K⁺ recycling which is an important step in Na⁺ absorption. Human colonic epithelium dissected off freshly resected colorectum specimens was mounted in Ussing chambers. The transepithelial voltage was clamped to 0 V, and the short-circuit current (SCC) recorded. To study the basolateral membrane in isolation, the apical membrane was perfused by the ionophore nystatin (allows bi-directional passage of monovalent cations between the mucosal bath solution and the cytoplasm). A mucosa to serosa K⁺ gradient was imposed. Since nystatin pores are also permeable to Cl⁻ and Ca²⁺ ions – these were reduced in the apical bath to 20 mM and 10 mM respectively.

Post addition of nystatin, the SCC stabilised at 50–70 μA/cm². 91% of the K⁺ dependent SCC was inhibited by tolbutamide (100 μM) added to the serosal bath and the remaining basolateral K⁺ current was inhibited by tetratetramethionium (TPTA) (inhibitors of ATP regulated K⁺ channels (KATP) and Ca²⁺ activated K⁺ channels (KCa)) respectively. To determine how these channels are affected by intracellular pH and calcium, one was inhibited using either TPTA or tolbutamide and the variation in the SCC produced by the other channel was monitored while varying pH or calcium concentration in the mucosal bath. Activity of the KATP channel was increased by intracellular alkalisation and by a decrease in intracellular calcium. Current generated by KCa channels was increased by raising cytosolic Ca²⁺ and also showed maximum activity at pH 7.5. Diazoxide (100 μM) and aldosterone (100 nM) activate KATP channels in other tissues, and in colon both immediately increased the tolbutamide-sensitive serosal K⁺ conductance (18% and 38% respectively). Addition of amiloride (100 μM which inhibits Na⁺ : K⁺ exchange) to the serosal bath prevented the rapid aldosterone effect.

The dominant electronegative ion conductance in the serosal membrane is via Na⁺ and K⁺ channels and Ca²⁺ recycling is such an important step in Na⁺ absorption, the clearer understanding of their regulation and pharmacological inhibition/up-regulation provided here may in the future allow for manipulation of fluid and electrolyte transfer across the colonic epithelium.

1298 Colonic Lamina Propria Lymphocyte' Subsets in Acromegalic with Intestinal Polyps

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There are few informations about immunological assessment of gut epithelium in acromegalic patients. We evaluated lymphocyte' subsets into colonic lamina propria (CLM) of patients with acromegaly.

Three months preliminary data are reported. Eight acromegalic (2 w, 6 m, age 27–65 years) underwent total colonoscopy to detect polyps. GH and IGF-I levels were 20.4±17.5 and 328.3±130 ng/ml in the serum. Only 1 patient showed intestinal symptoms. During endoscopy all polyps were resected and histologically analysed, biopsies were performed in the polyps' surrounding areas to study CLM lymphocytes subsets. Immunocompetent cells were detected by tissue digestion and cytofluorimetric assay as previously described [1].

We observed polyps in 7 patients (1 tubulo-villous adenoma, 5 tubular adenomas and 3 hyperplastic polyps). CLM lymphocytes from acromegalic patients (pts) and 20 control subjects (cts) were compared as shown in the table (median).

<table>
<thead>
<tr>
<th>CD20</th>
<th>CD12</th>
<th>CD4</th>
<th>CD8</th>
<th>3-DR+</th>
<th>3-DR-</th>
<th>CD18</th>
</tr>
</thead>
<tbody>
<tr>
<td>pts 52.0</td>
<td>8.2</td>
<td>37.6</td>
<td>13.5</td>
<td>8.6</td>
<td>5.9</td>
<td>10.0</td>
</tr>
<tr>
<td>cts 52.0</td>
<td>8.2</td>
<td>37.6</td>
<td>13.5</td>
<td>8.6</td>
<td>5.9</td>
<td>10.0</td>
</tr>
</tbody>
</table>

In conclusion no significant correlation between acromegalic and CLM lymphocyte subsets was observed in this preliminary report, also regarding polyps in the colon.


1299 Phenotypic Analysis of Peripheral T Cell and Phagocyte Alterations in Patients with Inflammatory Bowel Diseases


Several literature data suggest the immune system involvement in the Inflammatory Bowel Diseases (IBD) as documented by accumulation of activated lymphocytes in intestinal mucosa. Moreover an oligoclonality of Vβ TCR repertoire and an increased number of circulating gamma/delta TCR+ lymphocytes have been found in IBD patients. It is well documented that TCR heterodimers may be involved in MHC and non-MHC restricted cytotoxic activities. The aim of our study was to examine the level of peripheral blood gamma/delta TCR+ lymphocytes and CD56+ cells recognizing NK phenotype in patients suffering from IBD. 43 patients, 25 Ulcerative Colitis (UC) and 18 Crohn's Disease (CD), were compared to 11 normal controls.

Our results demonstrated a significant increase of gamma/delta TCR+ cells in UC (6.9±4.5% vs. 2.8±0.8; p = 0.005) and in CD (6.4±6.4; p = 0.017), with a similar rise of the absolute number in UC (92±70 vs. 42 ±19; p = 0.028) and in CD (75±43; p = 0.024). CD56+ cell percentages were equally increased in UC (23.5±11.4 vs. 15±4; p = 0.025) and in CD (25.3±30.2; p = 0.005). In UC the absolute number was 435±219 vs. 252±96 (p = 0.012), and in CD was 424±167 (p = 0.005). No differences were found between the two diseases. When results were analyzed according to the disease activity, no significant differences were observed between active and remission phase.

We suggest that the expansion of potentially cytotoxic cells in peripheral blood during IBD may derive, at least in part, from inflamed intestinal mucosa and may contribute to tissue damage.

1300 Gliadin Allergy with Chronic Urticaria and Atopic Eczema

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Introduction: The prevalence of allergic diseases with dermatological manifestations, as well as atopic eczema (A.E.) and chronic urticaria (Ch.U.) is gradually enhancing. Different food allergens can play important role in the pathogenesis of these diseases. Milk, egg, fish and cereal proteins are the most frequent food-allergens. The putative role of the most important cereal protein (gliadin) is poorly investigated.

Aim of the study: to examine the putative role of gliadin in the pathogenesis of A.E. and Ch.U.

Patients and methods: 10 patients with A.E., Ch.U. underwent detailed dermatological and immunological examinations. The local treatment of the cutaneous lesions and the oral administration of antihistamines remained unsuccessful in these patients. The following immunological parameters were measured in the blood: (1) gliadin specific Ig antibodies, (2) serum IgE level and/or Prick epicutan skin test, (3) the gliadin specific leukocyte migration inhibition (LMI) test. The mucosal mast cells' number (MMC) was analysed from endoscopic biopsies of the duodenum mucosa.

Results:

<table>
<thead>
<tr>
<th>Patients</th>
<th>LMI</th>
<th>IgG-AGA</th>
<th>IgE</th>
<th>Prick-test</th>
<th>MMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 A.E.</td>
<td>7 /10</td>
<td>4 /10</td>
<td>6 /7</td>
<td>3 /7</td>
<td>7 /8</td>
</tr>
<tr>
<td>10 Ch.U</td>
<td>8 /10</td>
<td>4 /10</td>
<td>3 /3</td>
<td>5 /10</td>
<td>7 /10</td>
</tr>
</tbody>
</table>

These results show that in the majority of the patients gliadin sensitized lymphocytes are present in the blood, while the level of the antigen specific antibodies remains less enhanced. The increased number of MMC in the intestinal lamina propria may represent a local immunoregulatory defect in the patients with A.E. and Ch.U. Six month later after the gliadin elimination from the diet the intensity of the skin lesions abated or disappeared.

Conclusion: The gliadin – similar to other nutritional allergens – can play an important role in the pathogenesis of A.E. and Ch.U. The LMI-test is more reliable test for the diagnosis of gliadin sensitivity. In atopic cases the conventional dermatologic therapy may be ineffective without the elimination of gliadin from the diet.

1301 Circulating Bacterial Lipopolysaccharides (LPS) and Evidence of Peripheral T Cell and Phagocyte Alterations in Patients with Inflammatory Bowel Diseases (IBD)

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LPS are the main components of the cell-wall from gram-negative bacteria endowed with different immunological activities. Since in patients with ulcer-
ative colitis (UC) and Crohn’s disease (CD) LPS have been detected in plasma in large amounts, the question arises on their origin and possible relationship with the host immune response. In this framework, we have evaluated the cellular immune responsiveness in a group of patients with IBD (15 UC and 10 CD subjects) under steroid treatment. The following immune parameters were evaluated: (1) Phagocytosis and killing of Candida albicans exerted by polymorphonuclear cells (PMN) and monocytes; (2) Antibacterial activity displayed by CD4+ and CD8+ cells using Salmonella typhi as target.

Results show that in all patients PMN and MO phagocytosis and killing and T cell function were significantly depressed when compared to normal donors. Plasmatic levels of LPS, detected by limulus assay, were found in 60% of both UC (24.4 ± 7.3 pg/ml) and CD (35.3 ± 7.5 pg/ml) patients. No LPS were detected in plasma from healthy blood donors.

In conclusion, it is conceivable that the observed reduction of phagocytic and T cell functions may allow the spread of exogenous and endogenous gram-negative organisms into the host, whose LPS, in turn, trigger the production of inflammatory mediators. Therefore endotoxaemia and peripheral cellular immune deficits may represent two possible related parameters which need an accurate monitoring in the course of IBD.

## 1302 The Expression of MHC Class-I Antigens in Gastric Cancer and Autologous Mucosa

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Class I molecules are required in the presentation of neoantigens on tumour cells to the cytotoxic T-lymphocytes. Their absence from the cell surface may lead to escape from immune surveillance. The altered expression of class I antigens has been reprotoed in the colon, breast and cervical cancers but in gastric cancer these are conflicting claims.

The objective of the present study was to evaluate the role of alterations of class I antigens in gastric cancer development. MHC class I antigens expression was analysed immunohistochemically in 50 primary gastric carcinomas from early to metastatic disease; autologous mucosa adjacent to carcinoma plus obtained stomach tissue from noncancer patients (n = 30). Snap frozen material and a monoclonal antibody W6/32 were used.

Only 2 out of 50 gastric carcinomas (4%) completely lost MHC class I antigens. The remaining tumours presented normal expression of this molecules. Interestingly, in 44% of patients, gastric epithelial tissue adjacent to the cancer and remote from the cancer showed the reduction of MHC expression. A loss of these antigens in nonneoplastic tissue was irrespective to tumour stage and grade. Furthermore this phenomenon was observed in intestinal metaplasia, atrophic gastritis as well as in normal gastric epithelium.

Epithelial tissue from all patients with benign gastric disorder and normal gastric tissue showed uniform immunopositivity with w 6/32 antibody.

The results obtained from current study suggest that alterations of MHC class I antigens in gastric epithelium may play an important role in the natural history of stomach cancer development.

## 1303 HLA Antigens in Ulcerative Colitis and Primary Sclerosing Cholangitis

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The purpose of the study was to find out whether the alleles of HLA class I or II region were associated with susceptibility to ulcerative colitis (UC). A second purpose was to show, whether there is a difference or similarity in HLA associations between primary sclerosing cholangitis (PSC) and ulcerative colitis.

HLA-A, B, C and DR antigens were studied using the standard lymphocyte microcytotoxicity test in 24 patients with PSC, 77 patients with UC and 106 controls.

HLA-B8 (54%) and DR3 (80%) were associated with PSC. A secondary association between DR3 and PSC was found. HLA-DR1, DR2 and DR6 were more common in UC than in controls. HLA-DR1 was the most common DR antigen in UC (46%). A positive association with Cw7 was common to both UC (25%) and PSC (33%). DR4 was underrepresented and DR6 overrepresented in both UC and PSC.

Our results indicate that ulcerative colitis is heterogeneous in its HLA associations and lacks the typical haplotype predisposing to autoimmunity. Primary sclerosing cholangitis and ulcerative colitis seem to be genetically separate diseases, despite some HLA associations in common.

## 1304 Mechanism of Immune Escape in Immunologically Heterogenous Tumours

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Tumours of the gastro intestinal tract evoke variable immunologic responses and are heterogeneous in terms of antigen spectrum and expression. Immune system reactions to such tumours would eliminate only the strongly immunogenic cells and allow the continued development of tumours which are weakly antigenic. To test this hypothesis we developed an animal tumour model, using tumour cells of different immunogenicity derived from the same original cell line. The SV3T3 cell line expresses a strong viral tumour specific transplantation antigen (TSTA). This particular cell line is tumourigenic in the athymic nude mouse but not in the immune competent animal. For weakly antigenic cells we used a JBS cell line which is a mutant strain derived from the original SV3T3 cells. This line was originally developed in our laboratory and is tumourigenic in the competent mouse and can be targeted by Cytotoxic T lymphocytes. Tumours were induced in immune competent mice by mixing equal numbers of the parental SV3T3 cells and the JBS tumour cell line. All animals developed palpable tumours which were fully vascularised. Immunohistochemical study of these tumours at 16 days showed them to contain both cell types – SV3T3 and JBS. At this stage there was a weak T cell infiltrate in the tumour. After sixteen to twenty days, the tumours reduced in size, but subsequently continued to grow between 30 and 45 days. Between 30 and 45 days the tumour infiltrate were predominantly polymorph nuclear leukocytes. Between 30 and 45 days there was absence of a tumour infiltrate and all the SV3T3 (strongly immunogenic cells) had been eliminated. Tumours continued to develop in 10 of 12 mice and were only of the JBS cell type.

These data indicate that the immune system selectively removes only strongly antigenic tumour cells from solid tumours. Immunologic elimination of weak antigenic tumour cells will require specific cell targeting. The recruitment of a potential immune reaction to a tumour by strongly antigenic cells does not non specifically eliminate the weak antigenic tumour cells. Immunotherapeutic strategies based on artificial augmentation of tumour cell antigens by viruses or chemical means may not result in targeting of weak antigenic cells.

## 1305 Anti-Neutrophil Cytoplasmatic Antibodies (ANCA) in Chronic Inflammatory Bowel Diseases

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Recently, anti-neutrophil cytoplasmatic antibodies (ANCA) were detected in sera of patients with chronic inflammatory bowel diseases. However, the frequency and subspecificity of ANCA in ulcerative colitis (UC) and Crohn’s disease (CD) are still a matter of discussion.

Therefore, we studied 32 sera from patients suffering from UC (12 p), CD (4 p) and nonspecific colitis and enteritis (6 p). As a control groups we also studied 32 patients with chronic glomeronephritis and 15 healthy persons. The disease activity was assessed according to Ruttgert’s and Maier’s criteria. Most of the patients with UC were treated with Salazopyrin and Captopril. The sera were tested on ethanol-fixed granulocytes and Hep2 cells to distinguish perinuclear fluorescence pattern (pANCA) from sera with anti-nuclear antibodies.

Two of the patients with UC (16%) were pANCA-positive and non of the rest, including the control groups showed any positive pattern. The ANCA (+) patients had the disease history more than 10 years with very often recurrence episodes despite the treatment and highest scores according to Maier’s criteria.

Our preliminary study proved the association of ANCA with active and severe UC and with other colon diseases. It remains to be investigated the incidence and pattern of ANCA in a bigger groups of UC and CD patients as well as the nature of the target antigens.

## 1306 Age-Associated Change in TH1 Like and TH2 Like Helper T Cells in the Peyer’s Patch of Senescence-Accelerated Mice

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The senescence-accelerated mouse (SAM) is a murine model of accelerated senescence, and previous studies have shown that immunological abnormalities and helper T cell dysfunction develop in SAM1P(SAMP1/Kyj) mice with aging. Recent studies indicate that anti-murine CD45RB monoclonal antibody (16A) separates helper T cells into two subpopulations, i.e., the high density...
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population containing normal TH1 like helper T cells which produce mainly IL-2 and not IL-4, and the low density population contain normal TH2 like helper T cells product mainly IL-4 and not IL-2.

To investigate the age associated change of these helper T cell subpopulations, two-color fluorescence flow cytometry was performed by incubating lymphocytes from the Peyer’s patch (PP), the spleen, and the peripheral lymph node (PLN) of SAMPI/Kgy and control SAMRI/Kgy mice with fluorescing anti-CD4 and 16A.

Lymphocytes from the Peyer’s patch and the spleen of SAMPI mice had decreased 16A-high density populations and increased 16A-low density populations in CD4 positive cells with age, but there was no change detected in lymphocytes of the peripheral lymph nodes.

Thus, these changes play a role affecting mucosal immunity of aging in SAMPI mice.

1307 Quality of Cholecystectomy Before and After Introduction of Laparoscopic Technique

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To evaluate the quality of cholecystectomy and establish a standard for continuing surveillance, data on all cholecystectomies (laparoscopic as well as conventional open procedures) were collected prospectively during the first two years after introduction of the laparoscopic technique. For comparison, data on all cholecystectomies in the last five years before laparoscopy were collected retrospectively with analysis of detailed case notes.

Parameters of quality included morbidity, 30-days mortality, reoperation rate and frequency of residual bile duct stones.

In the pre-laparoscopic period, 463 patients had open cholecystectomy. 174 of these had an acute operation. In the laparoscopic period (222 patients), 153 patients underwent laparoscopic cholecystectomy while 64 had a primarily open procedure. Thirty-seven of these had an acute operation. Thirteen patients (8.2%, 95% CI 4.4-13.5%) were converted from laparoscopic to open cholecystectomy.

The overall complication rate was reduced from 30% to 23% due to a reduction of general complications (17% vs. 10%, P < 0.05), mainly attributable to a reduction in the occurrence of pulmonary complications. The procedure related complication rate was 13% in both periods; septic complications dominating in the pre-laparoscopic period, while seven cases of bile leakage in the laparoscopic group, ductus reliance in the pre-laparoscopic period, while six cases in the ductus without wound dehiscence in the primarily open procedures dominated in the laparoscopic period. Bile duct injury occurred in 3/463 (0.6%, 95% CI 0.1-4.1%) patients in the pre-laparoscopic period, and in 2/222 (0.9%, 95% CI 0.2-2.1%) in the laparoscopic period (NS). Both cases occurred in the laparoscopic group.

The mortality rate was 1.5% and 0.5%, respectively (NS). Reoperation was necessary in 2.8% and 2.3% (NS), while residual stones were later detected in 1.5% and 2.7% (NS).

In conclusion, both general and procedure-related complications should be further reduced. This may be accomplished if more patients are operated laparoscopically, thus avoiding complications such as wound complications. Bile leak may be avoided through meticulous technique and increasing experience.

1308 Central Wire Rupture in Mechanical Lithotripsy of Bile Duct Stones – A New Technique in the Management of this Complication

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Introduction: While the break of one branch of the lithotripter basket allows in general the removal of the defective basket the complete break of the central wire lead to an impaction of the stone in the duct with non removable basket. Very often a surgical intervention is in those cases the only solution of the problem.

Patients: In a series of n = 535 patients with bile duct and pancreatic duct stones in n = 58 patients (10.6%, 2x pancreatic duct stones) a mechanical lithotripsy was necessary. We used the "Wurbs" system (Pauldrach Inc. Han- nover, FRG). In 3 of the 58 patients (5.2%, n = 2 bile duct stones and n = 1 pancreatic duct stone) the technical complication of the break of the central wire at the winch due to the hardness of the stone occurred.

Results: We tried first by insertion of a second basket system a lithotripsy and by this an excorporation of the first basket that was not successful. We solved the problem in the two cases of bile duct stones by guiding the broken cable once more through 10 cm shortened versions of the outer helix (specially made) and fixing them in the winch. By graduation in four steps of the spiral helix lengths of 10 cm (70-60-50-40) there was a four-times re-pretation of the lithotripsy possible. A 15 x 12 mm big pancreatic duct stone was due to his hardness not successfully extractable. Only after further application of extracorporeal shock waves the disaggregation of the stone and exorporation of the lithotripter basket was obtainable.

Conclusions: The system of the fourfold graduated length of the outer helices allows a new lithotripsy for three times in case of broken central wire. It should be available and present for every mechanical lithotripsy. With this device this severe technical complication is kept under control except the hardest stones without any surgical intervention. ESWL should be tried for hard stones in case of failed mechanical lithotripsy and sturred basket before a surgical intervention.

1309 Success of Mechanical Lithotripsy of Bile Duct Stones – Results in an Unselected Series of 515 Patients

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Introduction: In a series of 515 patients we examined the value of endoscopic stone removal from the bile duct system only by mechanical lithotripsy. In the literature at present there is 92% success rate by ESWL.

Methods: In patients with cholelithiasis we aimed at a complete sphincterotomy. Independent of the size of the stone, number, consistency, localisation and anatomic situation (e.g. Billroth-II-situs or duodenal diverticulum) we primarily tried to extract the stones with the basket. When there was a potential disproportion between possible sphincterotomy size and stone size and it was difficult to extract the stones the lithotripsy with the "Wurbs"-lithotriptor was performed.

Results: In the time from 09/90 to 11/93 we found in 2614 endoscopic retrograde cholangiograms (= ERC) n = 515 patients with bile duct stones, n = 103 patients. With large juxtapapillary duodenal diverticula, n = 304 patients of them had surgical indications, n = 211 multiple stones. At maximum we found in 1 pt. 25 stones. The diameter of the largest one was 4 cm. In n = 459 out of 515 patients (89.1%) a complete clearing of the bile duct system was possible with endoscopic sphincterotomy and stone extraction. In n = 56 out of 515 patients (10.9%) a lithotripsy was necessary. In n = 41 patients one session was sufficient. In n = 15 patients several procedures (in the mean 2.3 lithotripsy sessions) were required. Despite lithotripsy in n = 5 patients a stone elimination could not be completely performed. There were 3 patients who refused further endoscopic sessions and 2 patients with difficult anatomical situations (e.g. Billroth-II-situs or duodenal diverticulum).

Conclusions: In 51 out of 56 patients (91.1%) a mechanical lithotripsy was successful. In total we could reach a complete stone removal from the bile duct system by endoscopic therapy by mechanical lithotripsy in 510 out of 515 patients (99.0%) independent of stone size, consistency, localisation and the special anatomic situations by previous operations or diverticula.

1310 Extracorporeal Shock-Wave Lithotripsy of Gallbladder Stones – An Evaluation of Selection Criteria


Diverging results have been reported regarding the outcome of extracorporeal shock-wave lithotripsy (ESWL) of single and of multiple stones. Some of these differences may be explained by variations in selection criteria. With the introduction of laparoscopic cholecystectomy it has become even more important to define appropriate inclusion criteria for ESWL. Furthermore, it has been shown that the cost-effectiveness of ESWL for large stone burdens can not be compared with that of cholecystectomy.

Methods: Since 1989 we have treated 159 patients with ESWL (Dornier MPL 1000 lithotriptor) and oral litholysis (ursodeoxycholic acid) for symptomatic calcified or non-calcified gallbladder stones occupying at most half of the gallbladder volume. In 14 patients further treatment is planned to achieve satisfactory stone fragmentation which was defined as a maximum fragment size of 5 mm. The remaining 145 patients, which are analysed here, are either stone free, cholecystectomized, or have one or more remaining fragments of 5 mm or less.

Results: The overall cumulative gallbladder clearance rate was 11% at one month, 25% at six months, 40% at one year, 47% at two years, and 51% at three years. Corresponding figures for cholecystectomy were 1%, 7%, 16%, 32%, and 30%. Patients with a stone volume of ≤4.2 ml (corresponding to one spheric stone with diameter 2 cm) had significantly higher clearance rates than patients with a stone volume of ≥4.2 ml (16% vs 6% at one month, 33% vs 13% at six months, 53% vs 28% at one year, 59% vs 42% at two years, and 69% vs 36% at three years). Clearance rates were also higher for one stone than for multiple stones, and better clearance rates were achieved with a good than a poor gallbladder filling at oral cholecystography. In this series, the occurrence of calcified stones did not influence clearance rates. The gain in clearance rates due to selection according to other factors than stone volume was marginal. Patients with a stone volume of ≤4.2 ml constituted 13% of all