

Clinical Research in Gastroenterology 1

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edited by

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CLINICAL RESEARCH IN GASTROENTEROLOGY

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Preface

The last decade has seen tremendous developments in many fields of gastroenterology and hepatology. The aim of this series is to highlight some of these topics that deserve particular interest.

Research in the field of viral hepatitis has been very intense and successful in recent years. The hepatitis B virus is one of the best explored at the current level of virology. Not only the nucleotide sequence of the viral DNA can be decoded, but also the amino acid compounds of its genetic products are known today. Since the techniques of molecular biology have increasingly found access to clinical laboratory use, hepatitis B virus infection can serve as an example for the importance of molecular biology in clinical hepatology.

Another example for the interdependence of basic science and clinical medicine represents the research on bile acid metabolism. The investigation of bile acids has revealed new diagnostic approaches to hepatic and intestinal disorders. Commercial kits for the routine measurement of serum bile acids in clinical laboratories by enzymatic or radioimmunologic techniques are now available. The diagnostic value of these measurements in gastroenterology and hepatology shall be defined. Another aspect of bile acid research leads to new perspectives in the treatment of gallstone disease. The dissolution of cholesterol gallstones by chenodeoxycholic acid (therapy) may be quoted as the best example for the development of new pharmacotherapeutic principles derived from basic bile acid research. At present many therapeutic agents are used to dissolve gallstones not only in the gallbladder but also in the biliary tract. Therefore recent

advances on formation and dissolution of biliary calculi will be discussed.

Another field of rapid development in recent years has been that of operative endoscopy and endoscopic ultrasound. Endoscopic laser therapy has been used in the treatment of gastrointestinal haemorrhage. It is well established in the therapy of bleeding from peptic ulcers and might be of use in angiomatous lesions as well. Finally laser therapy finds more and more clinical application in palliation of gastrointestinal neoplasia and malignancies. In only five years the concept of laser treatment has advanced from a pure research idea to routine use in the management of gastrointestinal conditions. For this reason its place in clinical gastroenterology is indicated. In spite of recent progress in standard diagnostic imaging procedures, the combination of a gastroscope with an ultrasonic probe seems to open new perspectives for the examination of the oesophagus, the stomach, the duodenum and their neighbouring organs. Endoscopic ultrasound could become an important complementary diagnostic method in disorders confined to the gastrointestinal wall, the pancreas and the common bile duct, with particular sensitivity to the papilla of Vater. Therefore the value of this new technique will be reviewed.

Finally I would like to thank those who have participated in this volume for their excellent contributions on their individual fields of expertise. I am indebted to Mrs. Valerie Baker and Dr. Peter Clarke from MTP Press without whose assistance and understanding the organisation of this book would not have been possible.

Siegfried Matern

1

Clinical Significance of Molecular Studies of Hepatitis B Virus Infection

H. E. Blum and W. Gerok

INTRODUCTION

Infection with the hepatitis B virus (HBV) is endemic throughout much of the world with an estimated 200 million persistently infected people¹⁻⁵. HBV infects only humans and certain non-human primates, and belongs to a group of *hepatotropic DNA* (hepadna) viruses that includes the hepatitis virus of the woodchuck⁶, the Beechey ground squirrel⁷, and the Pekin duck⁸. In humans, HBV infection is associated with a wide spectrum of clinical presentations, ranging from the clinically inapparent 'healthy' carrier state, to acute and fulminant hepatitis, to various forms of chronic liver disease and to liver cirrhosis. Further, HBV infection is clearly involved in the development of hepatocellular carcinoma (HCC), worldwide a leading cause of death from cancer⁹⁻²⁵.

Important advances have been made during the past few years in the study of the biology of HBV and the virus/host cell interaction at the molecular level. The HBV genome has been cloned by recombinant DNA technologies and its detailed structure, including the complete nucleotide sequence, has been determined. The genetic organization of the genome has been established and viral genes have been expressed *in vitro* in various cell culture systems. Using cloned HBV DNA, viral

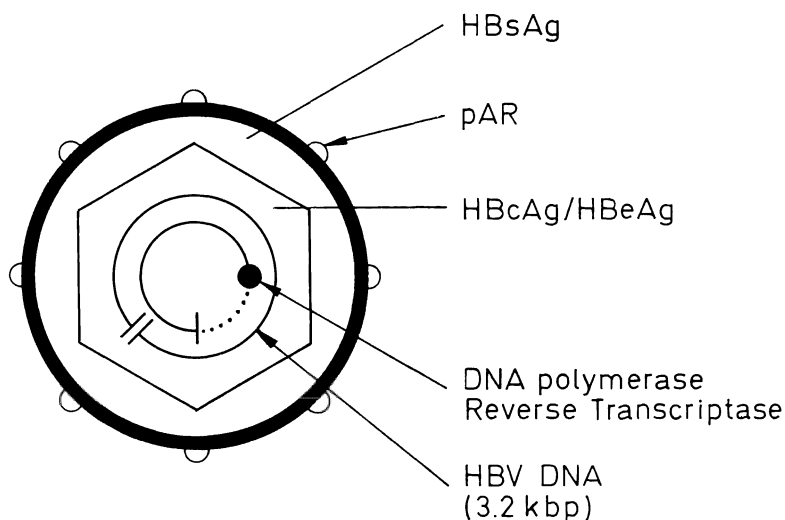


Fig. 1.1 Structure of hepatitis B virus (HBsAg, hepatitis B surface antigen; pAR, polyalbumin receptor; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; kbp, kilobase pairs)

nucleic acids have been identified and characterized in serum and liver of individuals infected by HBV using molecular hybridization techniques. These techniques have revealed two new and exciting aspects of the biology of HBV: the existence of HBV DNA in serum and liver of HBsAg-negative patients with liver disease (non-A, non-B hepatitis) and the presence of HBV DNA in non-hepatocytes. We will briefly review the structure, genetic organization and mode of replication of the HBV. Then we will describe recent developments in the laboratory diagnosis of HBV infection by molecular hybridization analyses and their significance for a clinical understanding of the pathogenetic mechanisms leading to HBV-induced liver diseases, including HCC.

STRUCTURE OF HEPATITIS B VIRUS

The hepatitis B virion ('Dane particle') has a diameter of approximately 42 nm²⁶. It consists of an electron-dense internal core structure (nucleocapsid) with a diameter of 28 nm and an envelope of about 7 nm thickness (Fig. 1.1). The envelope of the virion carries the hepatitis B

surface antigen (HBsAg) which shares antigenic determinants with the incomplete viral particles (22 nm spherical and filamentous empty envelope forms)²⁷. Three main serotypes of HBsAg (adw, adr, ayw) are commonly observed with a distinct worldwide distribution²⁸. Electrophoretic analysis of HBsAg revealed three proteins called the major, the middle and the large protein^{29,30}. These proteins occur in glycosylated/non-glycosylated forms and are encoded by the S gene, the pre-S2/S genes and the pre-S1/pre-S2/S genes, respectively (see below). In addition, the envelope carries a receptor for polymerized human serum albumin^{29,31} which is believed to mediate the attachment of HBV to hepatocytes⁵.

The nucleocapsid contains the hepatitis B core antigen (HBcAg) and its cryptic antigenic determinant hepatitis B e antigen (HBeAg^{32,33}), the viral DNA with a protein covalently attached to the 5'-end of the minus-strand³⁴, a DNA polymerase/reverse transcriptase^{35,36}, and a protein kinase³⁷.

STRUCTURE OF HEPATITIS B VIRUS GENOME

The HBV genome is a small circular DNA molecule of about 3.2 kilobase pairs length³⁸ (Fig. 1.2). The DNA is only partially double-stranded with a single-stranded region that varies in length between 15 and 60% of the viral genome in different molecules³⁹⁻⁴¹, displaying a preferred minimum length of 650–700 nucleotides⁴². The complete minus-strand is of constant length of about 3200 nucleotides and has a nick with a protein covalently attached to its 5'-end³⁴, preventing phosphorylation of the DNA by polynucleotide kinase. The 5'-end of the incomplete plus-strand is about 200–300 nucleotides downstream from the 5'-end of the minus-strand, thus creating a cohesive overlap that maintains the circular structure of virion DNA^{39,43-45}. DNAs of this size and structure, including the single-stranded region and a DNA polymerase that functions to fill in the gap by elongation from the 3'-end of the incomplete plus-strand^{35,36,39-41} have been found so far only in HBV and the other hepadna viruses of woodchuck⁶, ground squirrel⁷ and Pekin duck⁸.

The DNAs of all four hepadna viruses have been cloned in bacteria⁴⁶ and the complete nucleotide sequences of HBV⁴⁷⁻⁵⁰, woodchuck^{51,52} and Pekin duck^{53,54} DNAs have been established.

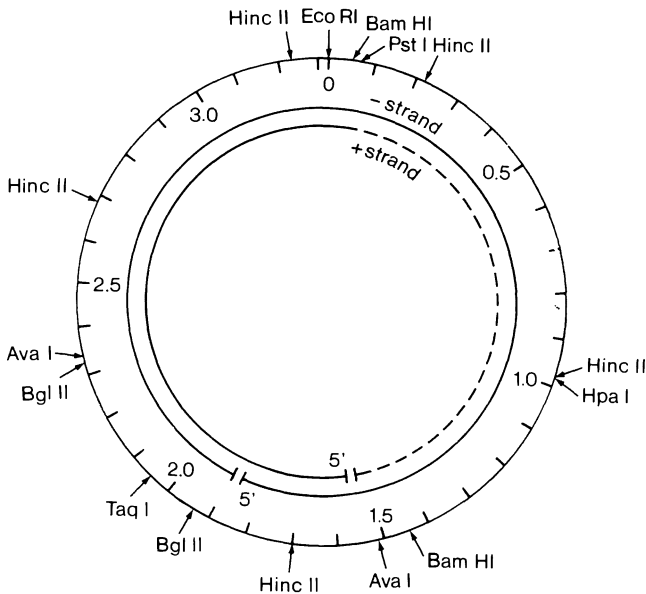


Fig. 1.2 Physical structure and restriction enzyme cleavage sites of the HBV genome (HBsAg subtype adw2⁴⁵)

GENETIC ORGANIZATION OF HEPATITIS B VIRUS GENOME

The minus-strand of virion DNA contains four open reading frames based on the three reading phases of the nucleotide sequence and the position of AUG start and TAA, TGA, or TAG stop codons. A comparison of the nucleotide sequence of the reading frames with the available information on the gene products HBsAg and HBcAg (molecular weight, amino acid composition and sequence) allowed the identification and localization of these viral genes in the viral genome (Fig. 1.3). These coding regions include the region that codes for the three proteins of HBsAg, including the receptor for polymerized human serum albumin (gene S and a contiguous region upstream termed pre-S gene), the region that codes for HBcAg (gene C) and the regions coding for DNA polymerase (gene P) and the X protein (gene X). Through extensive overlapping of gene P with genes pre S/S, X and C (Fig. 1.3) the small HBV genome can code for all known proteins: HBsAg (major, middle and large protein^{29,30}, HBxAg,

HBcAg/HBeAg and DNA polymerase/reverse transcriptase/RNA ase H.

REPLICATION OF HEPATITIS B VIRUS GENOME

Based on work with the Pekin duck hepatitis virus^{55,56} replication of HBV is strikingly different from all other animal DNA viruses and involves the reverse transcription of an RNA intermediate by a virus-encoded reverse transcriptase. This replication strategy is characteristic for the (RNA-containing) retroviruses, many of which are tumour viruses⁵⁷.

Upon internalization into host cells the infecting, partially single-stranded viral genome is made double-stranded, adopts a superhelical conformation and serves as template for the transcription of its minus-strand into viral RNA by a cellular RNA polymerase. These viral RNA molecules serve either as messenger RNA (mRNA) or as template ('pregenome') for the reverse transcription into minus-strand DNA. These reverse-transcribed minus-strand DNA species, synthesized by HBV reverse transcriptase, are then made partially double-stranded by the viral DNA polymerase to give mature virion DNA^{56,59}. Viral DNA replication is therefore asymmetric with separate pathways for minus- and plus-strand DNA syntheses and involves the reverse transcription of an RNA intermediate^{5,56,59}. In addition to the Pekin duck hepatitis virus^{55,56}, this mechanism has now been demonstrated to be operative also in ground squirrel hepatitis virus^{58,59} and the human/chimpanzee HBV⁶⁰⁻⁶³, indicating that this mode of replication is central to the life cycle of all hepadna viruses.

MOLECULAR HYBRIDIZATION ANALYSES

Molecular hybridization techniques, using cloned HBV DNA as a probe, allow quantitative and qualitative analyses related to the detection, characterization and localization of viral nucleic acid sequences in serum, cells or tissues. The principle of all hybridization analyses is the formation of a hybrid between nucleic acids from serum, cells or tissues and radiolabelled cloned HBV DNA through complementary base pairing. The radiolabelled hybrid molecules can then be detected by autoradiography.

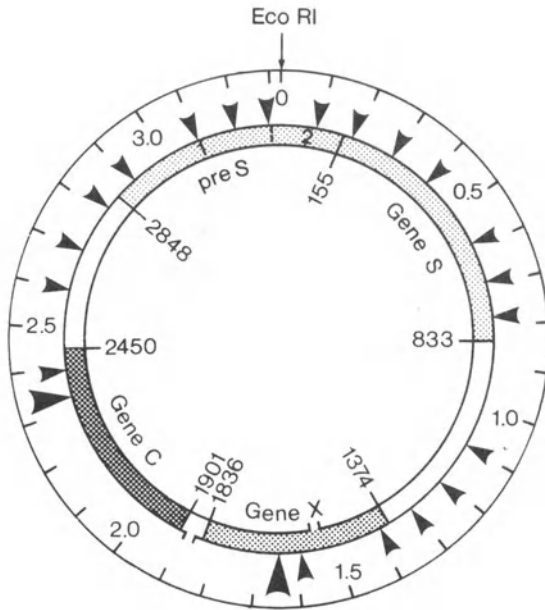


Fig. 1.3 Genetic organization of HBV genome and localization of HBV genes pre-S1, pre-S2, S, X, C and P (DNA polymerase/reverse transcriptase, arrows)

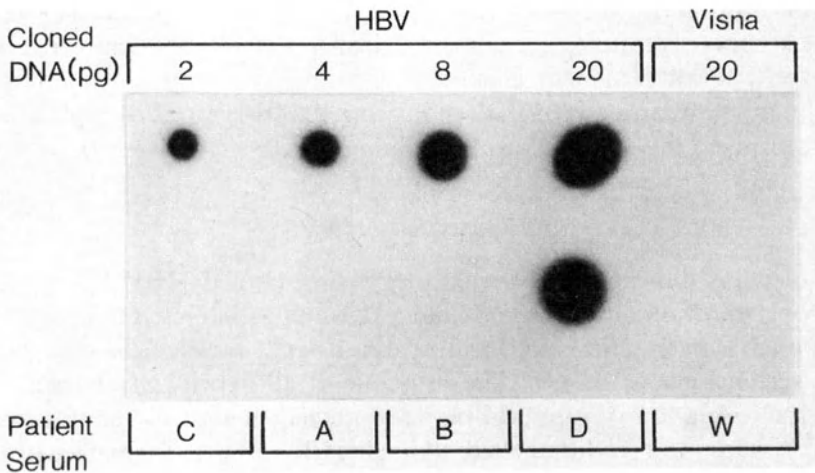


Fig. 1.4 Dot blot hybridization analysis of cloned HBV DNA, Visna cDNA and HBsAg-positive patient sera

Table 1.1 Hybridization techniques: detection and characterization of HBV DNA/RNA by dot blot, transfer blot and *in situ* hybridization

	<i>Dot blot</i>	<i>Transfer blot</i> (<i>Southern/Northern</i>)	<i>In situ</i> <i>hybridization</i>
DNA	+	+	+
RNA	+	+	+
Quantitation	+	+	+
Characterization:			
RNA size classes	-	+	-
DNA size classes	-	+	-
DNA conformation	-	+	-
DNA integration	-	+	-
Restriction analysis	-	+	-
Replicative forms (ssDNA)	+	+	+
Single cell analysis	-	-	+
Tissue pattern of viral DNA/RNA transcripts	-	-	+
Detection of rare/focal nucleic acid sequences	-	-	+
Combination of DNA/RNA hybridization with immunohistochemistry	-	-	+

These analyses offer a new insight into the biology of HBV in acute and chronic liver disease, as well as into the oncogenic potential of HBV as a cause of hepatocellular carcinoma. Three distinct types of hybridization assays are used, each of which has its particular power (Table 1.1): dot blot hybridization, transfer blot hybridization and *in situ* hybridization.

Dot blot hybridization

The principle of this technique is the immobilization of nucleic acids (DNA/RNA) from serum, cells or tissues on a membrane, followed by hybridization with radiolabelled cloned HBV DNA. Annealing of viral DNA/RNA to the HBV-specific probe results in radiolabelled hybrid molecules which can be detected by autoradiography and

quantitated by densitometry or liquid scintillation counting, relative to standards of known amounts of cloned viral DNA (Fig. 1.4).

The dot blot hybridization assay is specific for the detection of HBV DNA since an unrelated radiolabelled probe (e.g. pBR 322 DNA) or DNA from an unrelated virus (e.g. Visna cDNA) do not result in a detectable hybrid formation as illustrated in Fig. 1.4.

This assay is easy to perform and readily identifies HBV DNA in the serum from an HBV carrier, serologically positive for HBsAg, HBeAg and anti-HBc (patient serum D, Fig. 1.4). This result unequivocally demonstrates active viral replication in this patient's liver and is indicative for a high risk of transmitting HBV infection to others, especially to recipients of blood or blood products and to close personal contacts. In contrast, three patients serologically positive for HBsAg, anti-HBe and anti-HBc (patient sera C, A, B and W, Fig. 1.4) are negative for serum HBV DNA.

Transfer blot hybridization

The principle of this technique is the separation of DNA or RNA molecules according to size by electrophoresis through agarose, followed by transfer to a nucleic acid binding membrane under conditions preserving the relative positions of the DNA or RNA species in the gel. The immobilized DNA or RNA molecules are then hybridized with a radiolabelled HBV DNA probe. The presence and position of the nucleic acid species complementary to the probe is then detected by autoradiography. This technique was first described in 1975 for the analysis of DNA molecules⁶⁴ and popularly termed Southern blot analysis. In keeping with this terminology, the transfer blots for the characterization of RNA molecules were then termed Northern blots.

Southern blot analyses of DNA extracted from liver tissues have been extensively used in characterization of the state of HBV DNA in various forms of liver disease. These studies have demonstrated that HBV DNA in hepatocytes can exist in free, extrachromosomal, actively replicating forms as well as covalently integrated into the cellular genome.

The hybridization pattern of DNA extracted from the liver of a patient with active viral replication is characterized by the presence of DNA molecules of full length (3.2 kilobase pairs) and large amounts

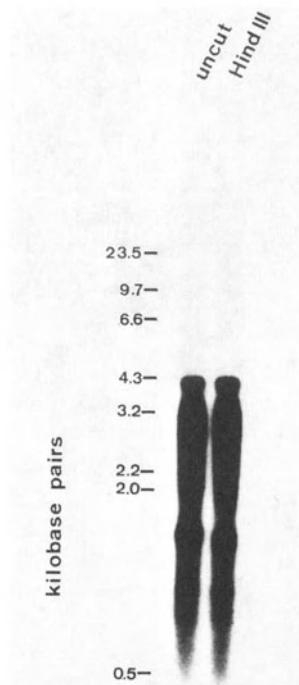


Fig. 1.5 Southern blot hybridization analysis of DNA extracted from the liver of a patient with HBeAg-positive chronic active hepatitis. Digestion with restriction endonucleases indicated on top

of smaller, nascent nucleic acids (Fig. 1.5). These species were shown to be predominantly single-stranded and of minus-strand polarity, demonstrating the asymmetric replication of the HBV genome (see above). By contrast, in hepatocellular carcinoma HBV DNA is predominantly integrated into the cellular genome. These integrated viral nucleic acid sequences are detectable only after digestion of cellular DNA by restriction endonuclease(s) (Fig. 1.6): as a result of the flanking chromosomal sequences the viral DNA species can have apparent lengths of more than 3.2 kilobase pairs, the unit-length of the free viral genome (see above).

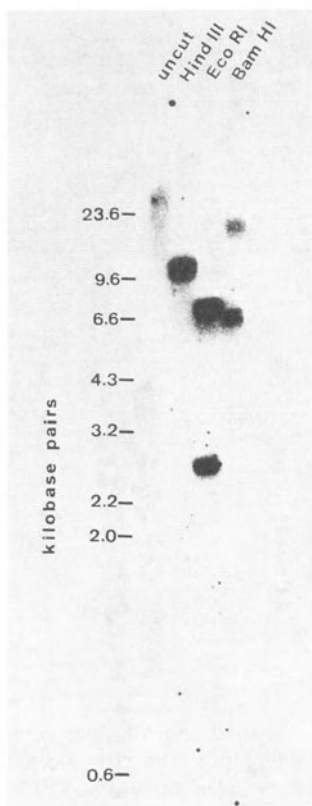


Fig. 1.6 Southern blot hybridization analysis of DNA extracted from a human hepatocellular carcinoma. Digestion with restriction endonucleases indicated on top

In situ hybridization

The principle of this technique is the annealing of single-stranded nucleic acid molecules in tissues, cells or cellular preparations (e.g. chromosomes) to a single-stranded, radiolabelled probe to form a hybrid molecule that can be detected by autoradiography. Because of the combination of molecular hybridization and cytological pro-

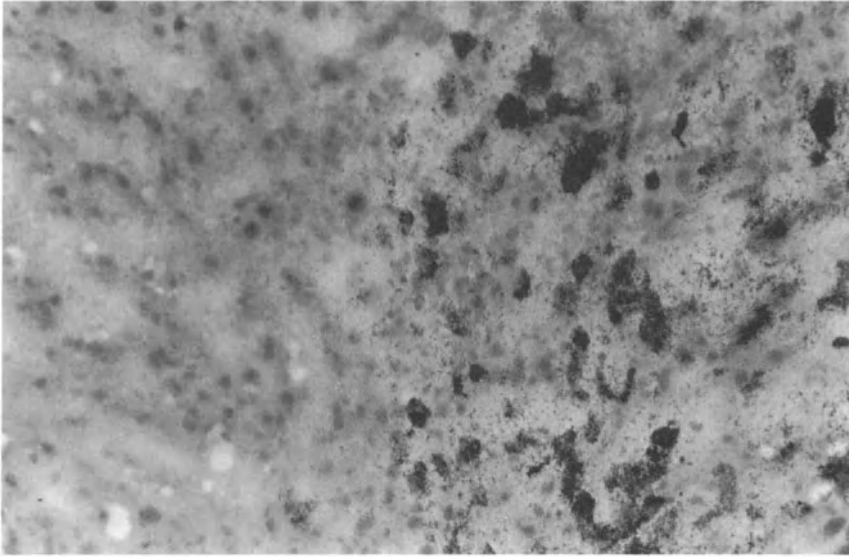


Fig. 1.7 *In situ* hybridization analysis of the liver from a patient with HBeAg-positive chronic active hepatitis.

cedures it is possible to localize genes on chromosomes, to follow their expression in cells and to detect and quantitate viral genes in infected cells.

By this technique HBV DNA is readily detected in infected hepatocytes and shows a highly focal tissue distribution with infected cells next to uninfected cells (Fig. 1.7). Patients with active viral replication display predominantly cytoplasmic hybridization patterns. These cytoplasmic viral nucleic acid species were shown to be mostly single-stranded and of minus-strand polarity, and demonstrate at the cellular level the asymmetric replication of HBV DNA⁶⁰.

CLINICAL SIGNIFICANCE OF MOLECULAR HYBRIDIZATION ANALYSES

Dot blot hybridization

The dot blot hybridization assays are specific for the detection of HBV DNA in serum, and viral nucleic acids from cells or tissues. This

technique is easy to perform and allows the simultaneous analysis of a virtually unlimited number of samples. If positive, dot blot hybridization unequivocally identifies individuals with high levels of infectious virus in serum, reflecting active viral replication in their liver.

However, due to the relative insensitivity of the dot blot hybridization technique (limit of detection about 0.05 pg HBV DNA, equivalent to 15 000 virus particles), negative results do not exclude the presence of infectious virus in serum or active viral replication in the liver. On the other hand, several studies have now established that dot blot analyses are more sensitive for the detection of HBV in the serum than radioimmunoassay for HBeAg and tests for DNA polymerase activity^{65-73,106}. Additionally, HBV DNA has been detected by dot blot analyses in some HBsAg-negative sera with and without antibodies to viral antigens^{69,74-76,106}. By contrast, no HBV DNA was detected in sera from patients with acute hepatitis serologically negative for HBsAg and positive for anti-HBc and anti-HBs or with acute hepatitis serologically negative for all viral markers (non-A, non-B hepatitis⁷¹). Nevertheless, these findings provide remarkable evidence that some HBsAg-positive/HBeAg-negative patients, and HBsAg-negative individuals with or without antibodies to HBV antigens (non-A, non-B hepatitis) may have active HBV replication in their livers and have to be considered potentially infectious. Dot blot hybridization thus provides a sensitive and direct means of detecting HBV in serum. These results redefine the classical serological patterns of HBV infection and are epidemiologically very important in identifying individuals with HBV DNA in serum, irrespective of their HBsAg, HBeAg or anti-HBe status (Tables 1.2 and 1.3), indicating a high risk of transmitting HBV infection to recipients of blood or blood products and to close personal contacts.

Dot blot hybridization can also be applied for the detection of viral DNA or RNA extracted from cells, tissues or subcellular fractions. As an analysis preliminary to a more detailed characterization of viral nucleic acid sequences by transfer blots, dot blot hybridization allows the specific demonstration of HBV DNA or RNA and the calculation of the average number of viral copies per ml serum or per cell.

MOLECULAR STUDIES OF HEPATITIS B INFECTION

Transfer blot hybridization

The presence and state of viral DNA/RNA in liver tissues have been studied in detail by transfer blot hybridizations. Southern blot analyses

Table 1.2 HBV DNA in serum from HBsAg-positive patients with chronic hepatitis (A⁶⁹ and B⁶⁷)

	<i>HBsAg</i>	<i>HBeAg</i>	<i>Anti-HBe</i>	<i>HBV DNA positive</i>
Hepatitis A	+	+	-	96% (52/54)
	+	-	+	63% (14/22)
	+	-	-	66% (2/3)
Hepatitis B	+	+	-	100% (10/10)
	+	-	+	54% (13/24)

Table 1.3 HBV DNA in serum from HBsAg-negative patients with chronic hepatitis⁷⁶

<i>HBsAg</i>	<i>Anti-HBc</i>	<i>Anti-HBs</i>	<i>HBV DNA positive</i>
-	+	+	3.8% (4/105)
-	+	-	5.7% (6/105)
<i>Total</i>			9.5% (10/105)

of DNA extracted from liver tissue have been extensively used in characterization of the state of HBV DNA in various forms of liver disease. These studies have demonstrated that HBV DNA in hepatocytes can exist in free, extrachromosomal forms as well as integrated into the cellular genome.

Free viral DNA has been generally found in the liver of HBeAg-positive patients^{12,60,62,63,70} while integrated forms are predominantly associated with HBe-negative chronic liver disease and HCC^{10,12,17,21-25,78}.

However, not all HCCs in patients with past or present HBV infection contain viral DNA integrated into the cellular genome^{79,80}.

On the other hand, HBV DNA has been demonstrated in the liver of some patients with HBsAg-negative liver disease with or without

Table 1.4 HBV DNA in non-hepatocytes

<i>Cells/tissues</i>	<i>Reference</i>
Bile duct epithelium, endothelial and smooth muscle cells of the liver	101
Spleen	102
Lymphoblastoid cells	83–85
Peripheral blood mononuclear cells	86–89, 93, 94
Pancreas, kidney, skin	90
Kaposi's sarcoma	95

antibodies to HBV antigens^{10,12,74–76,81}, but not in others⁸². These findings suggest that at least in some cases of serologically defined non-A, non-B hepatitis, the chronic liver disease may in fact be caused by HBV or HBV-related agents.

Southern blot analyses of liver DNA thus provide a powerful method for the detection and characterization of viral DNA species in various forms of HBV-related liver disease, including HCC, and permit the study of the interaction of the virus with the host cell at the molecular level. Further, HBV DNA has been demonstrated in cells other than hepatocytes (Table 1.4): in lymphoblastoid cells^{83–85}, peripheral blood mononuclear cells^{86–89,93,94}, pancreas, kidney and skin⁹⁰ of HBV-infected individuals. Also, in the chimpanzee and woodchuck models of hepatitis B, viral DNA was detected in peripheral blood lymphocytes⁹¹. HBV DNA has been further detected in saliva, urine and seminal fluid of carriers of HBeAg⁹². Finally, HBV DNA sequences were found in lymphoid cells⁹³ and in peripheral blood mononuclear cells⁹⁴ of patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex, and in Kaposi's sarcoma⁹⁵. All these findings indicate that the host cell range for HBV is more extensive than has been appreciated previously. The biological significance and pathological potential of the presence of HBV DNA in non-hepatocytes, however, remain to be defined.

In situ hybridization

At the tissue and single cell level viral nucleic acid sequences can be detected by *in situ* hybridization. *In situ* hybridization has been applied

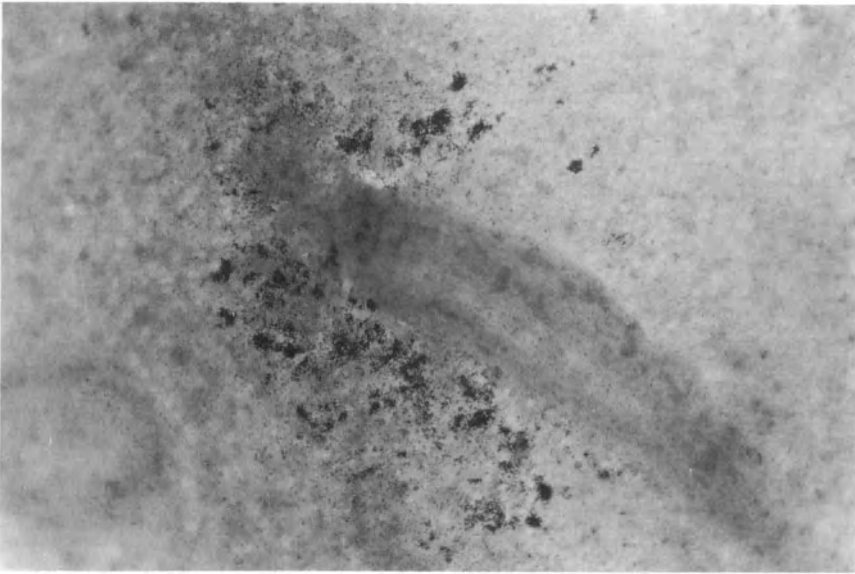


Fig. 1.8 *In situ* hybridization analysis of the spleen from a patient with HBeAg-positive chronic active hepatitis

for the study of HBV-related liver disease in tissues and HBV DNA containing cell lines⁹⁶⁻¹⁰⁵. The technique has proven to be specific for detection of HBV DNA or RNA since hybridization with an unrelated probe (e.g. pBR 322 DNA) or to uninfected human liver results in a negative hybridization signal. This assay is highly sensitive and detects as little as about 5 HBV genome equivalents per cell, as determined by comparative hybridization analyses of human hepatoma cells (PLC/PRF/5; Alexander cells) which contain about four copies of HBV DNA integrated into the cellular genome¹⁰¹.

Due to the high sensitivity of the *in situ* hybridization assay and visualization of the infected cells, HBV DNA could be demonstrated not only in hepatocytes but also in the bile duct epithelium, in endothelial cells and smooth muscle cells of blood vessel walls in HBV-infected liver as well as in the spleen^{101,102} (Fig. 1.8). This assay has identified new target cells for HBV infection, extending the known host cell range for HBV. These findings are of particular significance for the understanding of the biology of HBV and are consistent with

several recent reports demonstrating HBV in various non-hepatocytes (Table 1.4).

The *in situ* hybridization assay thus provides a specific and sensitive tool for the localization and quantitation of viral nucleic acids at the single cell level and for the identification of individuals with active replication of HBV DNA hitherto not possible with other nucleic acid hybridization analyses. This assay is particularly useful since it requires only a fraction of a liver needle biopsy obtained routinely for diagnostic purposes, and should permit following the natural course of HBV infection and selecting and monitoring patients undergoing antiviral therapies.

SUMMARY AND PERSPECTIVES

The HBV genome consists of a small circular, partially double-stranded DNA molecule of about 3200 nucleotides length. The physical structure and localization of the major genes of HBV have been identified and the complete nucleotide sequence of the HBV genome has been established. The viral genome replicates asymmetrically with separate pathways for the minus- and plus-strand synthesis and involves the reverse transcription of an RNA intermediate as a central feature of its replication strategy, characteristic for the (RNA-containing) retroviruses, many of which are tumour viruses⁵⁷.

HBV infection in humans is associated with a wide spectrum of clinical presentations, ranging from the clinically inapparent, 'healthy' carrier state, to acute/fulminant hepatitis, various forms of chronic hepatitis and liver cirrhosis to hepatocellular carcinoma. Currently, the specific laboratory diagnosis of HBV infection is based on the serological detection of HBsAg, HBeAg/anti-HBe, anti-HBc and anti-HBs. Three newly developed molecular hybridization techniques have permitted: (1) the direct identification of HBV DNA in serum by dot blot analysis; (2) the characterization of viral nucleic acid sequences with respect to their interaction with the host cells (free, extra-chromosomal vs. integrated viral DNA) by Southern blots; and (3) the localization and quantitation of viral nucleic acid sequences at the single cell level by *in situ* hybridization, combined with the immunohistochemical visualization of viral antigens¹⁰³. These analyses permit a comprehensive assessment of the biology of HBV at the

genetic (DNA), transcriptional (RNA) and translational (antigens/proteins) levels.

The clinical significance of the molecular analyses of HBV infection lies:

1. in the detection of active viral replication by demonstration and characterization of HBV nucleic acids in serum and/or liver of patients with HBV infection;
2. in the estimation of the infectivity of patients with HBV infection and their potential risk to others¹⁰⁶;
3. in the possibility of following the natural course of HBV infection^{71-73,107-109} and to assess the effectiveness of antiviral or immunosuppressive therapy¹¹⁰⁻¹¹⁸;
4. in the detection of integrated HBV DNA in hepatocellular carcinoma from patients serologically positive or negative for HBV markers, suggesting a pathogenetic role of HBV in the mechanisms leading to the malignant transformation of hepatocytes;
5. in the demonstration of HBV as an aetiologic agent in serum and/or liver from patients with HBsAg-negative liver disease, some of whom are negative for all markers of HBV infection (non-A, non-B hepatitis);
6. in the detection of viral nucleic acids in non-hepatocytes from patients with or without serological evidence of past or present HBV infection.

With increasing knowledge of the structural and biological characteristics of HBV we shall gain more insight into the pathogenetic mechanisms leading to acute and fulminant liver cell injury, to viral persistence and chronic liver disease, including hepatocellular carcinoma. While HBV replication does not seem to be directly cytopathic, several lines of evidence suggest that immune mechanisms are involved in the pathogenesis of HBV-associated liver cell injury¹¹⁹⁻¹²³.

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2

Clinical Value of Serum Bile Acid Determination

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It has been known for many years that an elevated level of serum bile acids is a highly sensitive and specific indicator of hepatobiliary dysfunction¹ and it seems that serum bile acid levels may give better insight into the functional state of the liver than the traditional liver tests^{2,3}. The availability of commercial kits for enzymatic and radio-immunological determination of serum bile acids made it feasible to determine serum bile acids in the routine clinical laboratory and to evaluate their clinical usefulness. For optimal use and interpretation of serum bile acid measurements it is of importance to know the physiological and pathophysiological determinants of serum bile acid levels.

PHYSIOLOGY OF BILE ACIDS

In healthy subjects the bile acid pool of about 6 mmol cycles 5–15 times daily through the enterohepatic circulation. This is accomplished by two physical pumps (gall bladder and small intestine), two chemical pumps (transport system of the hepatocyte and the terminal ileal enterocyte), and two sphincters (sphincter of Oddi and ileocaecal sphincter)^{4,5}. The bile acid pool consists of primary, secondary and tertiary bile acids. Primary bile acids are synthesized exclusively in the

hepatocyte from cholesterol involving enzymes of the endoplasmatic reticulum, the soluble compartment of the hepatocyte, the mitochondria and the peroxisomes^{6,7}. The first step in the formation of bile acids is the 7α -hydroxylation of cholesterol. The 7α -hydroxylase is the rate-limiting enzyme in the biosynthesis of bile acids^{8,9}. Subsequently a 3-keto-derivative is formed, which is the precursor of the two main primary bile acids in man: chenodeoxycholic acid and cholic acid. When the side-chain of this intermediate is cleaved after 26-hydroxylation, a propionic acid-CoA intermediate is released and chenodeoxycholic acid, a dihydroxy bile acid, results. Cholic acid, a trihydroxy bile acid, is formed when 12α -hydroxylation precedes cleavage of the side-chain. The rate of cholic acid synthesis is about twice that of chenodeoxycholic acid¹⁰. Under steady-state conditions daily synthesis of 0.5–1.0 mmol bile acids compensates for faecal loss.

The secondary bile acids are formed in the intestine by bacterial dehydroxylation. Under normal circumstances dehydroxylation starts in the distal section of the ileum and continues throughout the colon. *In vivo* the most frequent reactions are deconjugations and oxidoreductions of hydroxyl groups. The hydroxyl groups, however, differ in their chemical reactivity, the 7α -hydroxyl group being the most and the 12α -hydroxyl group the least reactive one so that 7-oxo-bile acids are formed predominantly¹¹. The major secondary bile acids in man are deoxycholic acid and lithocholic acid, dehydroxylation products of cholic acid and chenodeoxycholic acid, respectively. In healthy adults secondary bile acids represent about 20% of the total bile acid pool.

Tertiary bile acids are formed in the liver from secondary bile acids; an example is the formation of ursodeoxycholic acid from 7-ketolithocholic acid^{12,13}.

Bile acids are secreted by the hepatocyte as conjugates with glycine or taurine, in an average ratio¹⁴ of around 3 to 1. Two lysosomal enzymes, cholyl-CoA-synthetase and acyl-transferase, give rise to this conjugation^{14,15}. Conjugation alters the physicochemical and physiological properties of bile acids¹⁶. The amide bond has an electron-attracting property and increases the ionization of the carboxylic acid group in the glycine conjugates which have a pK of about 3.5. The sulphonic acid group of taurine is intrinsically quite strong (pK 1.5)¹⁷. At the pH in the gut conjugation increases the hydrophilicity of bile

acids and thus decreases their ability to cross the lipophilic layer of cell membranes. As a consequence, conjugated bile acids for the major part are not absorbed passively during their passage through the biliary tree or the small intestine. Most of the intestinal reabsorption of bile acid conjugates therefore occur by an active process in the terminal ileum¹⁸.

After excretion into the gut conjugated bile acids are subjected to deconjugation. The removal of the glycine or taurine conjugate is catalysed by strains of *Clostridium*, *Bacteroides*, *Enterococcus*, and *Lactobacillus*^{11,19}. Bacterial modification begins in the distal small intestine; however, most bile acids are absorbed from the small intestine before bacterial deconjugation occurs. Only a small fraction of bile acids escapes absorption in the small intestine and passes into the colon where it is biotransformed by bacteria¹¹. The pK of the unconjugated bile acids is higher than the intestinal pH. Consequently free bile acids are mostly non-ionized and are taken up by passive non-ionic diffusion. Trihydroxylated bile acids have a greater affinity to the transport site than dihydroxylated bile acids. The monohydroxylated lithocholic acid has a low solubility. Thus only a minor proportion of lithocholic acid is reabsorbed, contributing only a small fraction to the bile acid pool. In addition to conjugation, sulphation and glucuronidation may occur^{20,21}. Sulphation is induced by sulphokinase²² and glucuronization is carried out by UDP-glucuronyl-transferases^{23,24}. Conjugation with sulphate and glucuronic acid further increase water-solubility of the glycine and taurine conjugates. Sulphation seems to be the main detoxifying mechanism of lithocholic acid. Sulphate esters contribute 50–80% of total lithocholic acid found in the bile of normal persons²⁵. Under certain pathological conditions, especially severe cholestasis due to extrahepatic obstruction, hepatitis, cirrhosis, and tumours of the liver mono-, di-, and trisulphates of bile acids can be identified in the urine and sulphation becomes the main mechanism for elimination of bile acids^{21,26,27}. Similarly, glucuronidation of bile acids seems to be an escape mechanism facilitating excretion of bile acids during cholestatic liver injury. In patients with intrahepatic or extrahepatic cholestasis approximately 10–20% of the bile acids excreted in the urine were glucuronic acid conjugates²³. However, there were considerable individual differences, and in some patients investigated the proportion of the glucuronides exceeded

that of the sulphate esters²³. It has been demonstrated that UDP-glucuronyl-transferases can be induced by barbiturates²⁸.

The cycling frequency of the bile acid pool is in part modulated by filling and emptying of the gall bladder. During fasting most of the bile acid pool (70–80% at the end of the night) is present in the gall bladder²⁹. Gall bladder contraction in response to meals or pharmacological stimuli will increase the hepatic load of bile acids and consequently peripheral serum blood levels of bile acids. Absence of a functioning gall bladder reduces the postprandial rise of serum bile acid levels at least in the first year after surgery³⁰. After cholecystectomy the common bile duct can have a certain storage function if the sphincter of Oddi is intact and serum bile acid levels will still raise in the postprandial state although to a smaller extent⁵. The sphincter of Oddi has been supposed to be a regulator of the enterohepatic circulation of bile acids and of bile acid output into the duodenum independently of gallbladder function³¹. After feeding most of the bile acid pool is secreted into the duodenum. The enterohepatic circulation is influenced by the interdigestive intestinal motility which transports the bile acid pool to the terminal ileum. Increased intestinal motility with shortened intestinal transit time stimulates enterohepatic cycling frequency and decreases the size of the bile acid pool, whereas slowing the intestinal transit has an opposite effect³². Length and integrity of the small bowel affects the postprandial rise of serum bile acid levels^{33,34}. Bacterial overgrowth alters place and rate of bile acid absorption by deconjugation of bile acids³³ and leads to an increase of unconjugated serum bile acids³⁵.

In healthy subjects the fasting serum bile acid levels of chenodeoxycholic acid and deoxycholic acid are generally higher than those of cholic acid³⁶. This is due to the more ready uptake of dihydroxy bile acids compared to cholic acid in the proximal small intestine by passive absorption, and the more efficient hepatic uptake of cholic acid compared to chenodeoxycholic and deoxycholic acid^{37–39}.

DETERMINANTS OF SERUM BILE ACIDS

Serum bile acid levels at any moment are determined by the instantaneous balance between absorption of bile acids from the intestine and elimination of bile acids by the liver. Hepatic clearance of bile

acids occurs during their first hepatic passage from portal blood and after their recirculation from the systemic circulation both from portal and arterial blood. About 80–90% of conjugated trihydroxy bile acids are removed from portal blood during one passage by an active transport mechanism located in the sinusoidal plasma membrane of the hepatocytes. Extraction of unconjugated trihydroxy bile acids is somewhat less, namely about 70%. The values for conjugated and unconjugated dihydroxy bile acids are 70–80% and 50–60%, respectively^{40,41}. Spill-over of bile acids into the systemic circulation is the complement of these percentages: about 40–50% of unconjugated bile acids spill into the systemic circulation, whereas only 10–30% of the conjugated bile acids spill over. Despite this spill-over, less than 1% of the exchangeable bile acid pool is present in the blood compartment.

The transport mechanism responsible for hepatocellular bile acid uptake has a large excess capacity. Not only in healthy subjects but also in patients with mild to moderate liver disease, hepatic bile acid transport operates far below saturation^{42–44}. Therefore the fraction of bile acids extracted by the liver and its complement, the spill-over into the systemic circulation, remain nearly constant over the whole range of physiological bile acid loads to the liver. Thus intestinal absorption is the major determinant for the diurnal changes of the bile acid levels in peripheral blood. After meals the delivery of bile into the intestine, the flow of bile acids towards the terminal ileum and consequently intestinal absorption and the load of bile acids to the liver, increase. A greater load and a constant extraction fraction result in a greater load of bile acids which spills over into the systemic circulation and leads to the well-documented postprandial increase of serum bile acids. Total serum bile acid levels increases two- to five-fold after a meal⁴⁵. Chenodeoxycholic acid shows the most rapid and largest increase followed by deoxycholic acid and cholic acid. This is mainly due to the fact that chenodeoxycholic acid is absorbed more rapidly from the upper small intestine and is extracted by the liver less efficiently than cholic and deoxycholic acid. The total glycine/taurine ratio of 2.5 in the fasting state increases to a maximum value of 3.3 at 1–1½ hours postprandially and then declines. This shift in glycine/taurine ratio shows that the increase in glycine conjugates exceeds the increase in taurine conjugates in the early postprandial period⁴⁵.

CLINICAL APPLICATION OF SERUM BILE ACID MEASUREMENTS

Blood tests are employed in clinical gastroenterology for diagnostic or prognostic purposes. The diagnostic approach is to classify the disease of a patient and implies a binary yes/no answer. For this purpose the bile acid level, a continuous variable, is converted to a discontinuous variable by introducing discrimination limits separating positive from negative results. The prognostic assessment is usually based on an estimate of the severity of the disease when the diagnosis has already been established. Test results are then reported in quantitative terms.

In recent years the results of several studies in larger populations, including healthy subjects and patients affected by various well-documented hepatobiliary diseases, became available. These compare the diagnostic value of serum bile acid levels with that of conventional liver tests in terms of sensitivity, specificity and predictive value. Results on sensitivity and specificity of serum bile acid measurements for detection of liver disease are influenced by the analytical method used. Any method performed by the routine clinical laboratory has to be accurate, sensitive enough to detect bile acids at the picomole level, and simple in handling. Although gas-liquid chromatography with or without mass spectrometry fulfils the first two criteria it is technically too demanding, and thus will remain a research tool. This leaves essentially enzymatic methods and radioimmunoassays for clinical use.

For determination of bile acid concentrations in serum enzymatic methods are widely used and are commercially available⁴⁶. A variety of enzymes specifically cleaving hydroxyl groups in the 3 α -, 7 α -, or 12 α -position are in use⁴⁷. The photometric and fluorimetric assays seem to be rather insensitive.⁴⁷ However, sensitivity has been improved by methods using the fluorescence of redox indicators⁴⁸. The recent description of a bioluminescence assay capable of detecting bile acids in the picomole range^{49,50} has brought the sensitivity of the assays to the levels of the radioimmunoassays. In addition this method does not require expensive equipment and is quite simple to perform. By incorporating different enzymes into the assay the bioluminescence method also has the potential for measuring individual bile acids.

For sensitive detection in serum many radioimmunoassay techniques, with antibodies against the main bile acids, have been developed. Several of these are commercially available. The potential drawback for the smaller laboratory, namely the need for a liquid scintillation counter or a gamma spectrometer, has been overcome by the development of enzyme-linked immunoassays⁵¹. Disadvantages of the radioimmunoassays and the enzyme-linked immunoassays are their different specificities and cross-reactivities with other bile acids.

There has been a great deal of controversy as to whether the measurement of postprandial serum bile acids is more sensitive than that of a fasting value for detection of liver disorders. Several studies have shown that the postprandial serum bile acid concentration, which has been called the endogenous bile acid tolerance test, discriminates better between healthy subjects and patients with liver disease than the same measurement in the fasting state⁵²⁻⁵⁴, though not all investigators agree on this point^{55,56}. During fasting and after a meal the fraction of bile acids extracted by the liver during one pass should be nearly the same in healthy subjects and in patients with mild to moderate liver disease, because the hepatic uptake of bile acids in these patients is far below its saturation^{42,43}. Therefore one could expect similar diagnostic sensitivities of fasting and postprandial serum bile acids.

The conflicting results may be due to differences in the analytical sensitivity and accuracy of the different methods used for bile acid measurements. Consequently Mannes⁵⁷ compared fasting and postprandial serum bile acids in patients with cirrhosis of the liver and healthy controls using three methods: an enzymatic assay, a radioimmunoassay, and a gas-liquid chromatographic method. When the enzymatic assay was used the fasting serum bile acid level was a markedly less sensitive parameter (diagnostic sensitivity 79%) for detection of cirrhosis than the postprandial serum bile acid level (93%). When the radioimmunoassay (diagnostic sensitivity 93% for both the fasting and the postprandial state) or an appropriate gas-liquid chromatographic method was used (diagnostic sensitivity fasting 95%, postprandial 93%) fasting serum bile acids were at least as sensitive for detection of cirrhosis as postprandial serum bile acids. Festi⁵⁶ has recently shown that fasting serum bile acids are even more sensitive, and discriminate better between normals and patients with liver disease than postprandial serum bile acids. In theory fasting

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levels are probably more reproducible in the assessment of liver disease since the postprandial rise of bile acids is dependent on a number of highly variable extrahepatic factors such as gastric emptying, digestive hormone release, gall bladder contraction and intestinal motility⁵⁸.

Relatively little information is available on specificity, sensitivity and clinical utility of exogenous bile acid loading tests^{24,59}. Measurements of oral clearance have proven more sensitive than those of intravenous clearance in the detection of liver disease. This is perhaps explainable by theory: after direct application of bile acids into the vascular compartment the elimination from plasma is highly dependent on liver blood flow, whereas after an oral loading absorbed bile acids are subject to high first-pass extraction and elimination by the liver⁴¹. For example, when a substance such as a bile acid with a high hepatic extraction of 90% is given intravenously, a 10% reduction in extraction due to liver disease will cause a proportional 10% reduction in plasma clearance; however, if the substance is administered orally and is, like bile acids, nearly completely absorbed, the fraction of the dose reaching peripheral blood from portal blood will be increased from 10% to 20%. By this, a 10% reduction in hepatic extraction increases the spill-over of bile acids by 100%. This explains the disappointing results of intravenous bile acid clearance tests in the detection of liver disease⁶⁰.

Bile acid levels are influenced not only by hepatic first-pass elimination, however; they are influenced to a lesser extent also by the clearance of systemically circulating bile acids which reach the liver both via the hepatic artery and via the splanchnic circulation. Decreases in first-pass elimination from portal blood (systemic availability) and reductions of hepatic clearance of bile acids from the systemic circulation contribute to increased serum concentration in patients with liver disease³². In accordance with pharmacokinetic principles applied to high extraction compounds it may be assumed that changes in systemic availability and clearance have multiplicative rather than additive effects on serum bile acid levels⁶¹.

DIAGNOSTIC VALUE OF SERUM BILE ACID LEVELS

Important information on sensitivity, specificity and predictive value of serum bile acid levels comes from the study of Ferraris⁶². In the

detection of hepatobiliary disease fasting serum bile acids measured fluorimetrically by means of an enzymatic technique (cut-off level $8.4 \mu\text{mol/l}$) and aspartate aminotransferase (AST, cut-off 20mU/ml) exhibited similar sensitivity (78% vs. 74%), specificity (93% vs. 92%), and predictive value for a positive result (94% vs. 93%). Gamma-glutamyltranspeptidase (γ -GT, cut-off 28mU/ml) had a sensitivity of 75%, a specificity of 85% (significantly lower than serum bile acids) and a predictive value for a positive result of 89%. When the predictive value of a positive result, which was obtained in a gastrointestinal unit with a 60% prevalence of hepatobiliary disease, was corrected for the actual prevalence of hepatobiliary disease in the general population (estimated as 1000/100 000) the predictive value decreased to 10% for serum bile acids, to 9% for AST, and to 5% for γ -GT. Since serum bile acids and AST in a mixed group of patients with hepatobiliary disorders have very similar sensitivities, specificities and predictive values, it seems that for cost/effectiveness reasons AST is preferable when a single screening test for liver disease is used. The combination of serum bile acids with AST improves the predictive value of a positive result to 32% vs. 10% of serum bile acids alone in the simulated screening situation with an estimated prevalence of the hepatobiliary disease in the general population of 1%.

When the data of the study of Ferraris⁶² are broken down with respect to severity of liver disease, it becomes obvious that γ -GT was found to be more suitable than serum bile acids in detecting mild hepatobiliary disease (sensitivity γ -GT 62%, serum bile acids 48%, AST 49%). However, serum bile acids proved to be significantly more sensitive (93%) than γ -GT (82%), AST (86%), alkaline phosphatase (78%), and other routine liver tests in detecting severe hepatobiliary disease.

Using a highly accurate and specific mass fragmentographic technique Einarsson³⁶ evaluated the diagnostic value of serum bile acids in anicteric patients with suspected alcoholic liver disease. Whereas serum bile acids showed a high diagnostic sensitivity in cirrhosis of the liver, the measurement of serum bile acids did not discriminate patients with fatty liver from controls. It thus appears that serum bile acid determinations are not useful in the detection of mild liver disorders. Similar results have been presented by other groups using different techniques^{56,63,64}.

Mishler⁶⁵ compared the concentrations of alanine aminotransferase (ALT) and serum bile acids in blood donors and in recipients in whom either non-A, non-B or type B hepatitis developed after transfusion. The results demonstrate that elevated concentrations of ALT in the blood of donors in most instances were accompanied by normal concentrations of serum bile acids.

Should bile acid determination be introduced in clinical practice for diagnostic reasons, it will not be ordered as a single test but rather in combination with other tests of the conventional test battery. Using stepwise discriminant analysis Festi⁵⁶ compared a battery of liver tests (AST, ALT, γ -GT, albumin, γ -globulin, bilirubin, alkaline phosphatase, and prothrombin time), serum bile acids and combinations with respect to their capability to correctly allocate subjects into histologically defined groups. The percentage of correct allocations was 75% for conventional liver test battery, 70% for fasting serum bile acids and increased to 80% when conventional liver tests together with serum bile acids were considered. Thus serum bile acids may have diagnostic utility in combination with conventional liver tests because of reducing misclassifications.

CIRRHOSIS OF THE LIVER

It has been shown that patients with advanced cirrhosis of the liver have elevated fasting and/or postprandial bile acid levels in serum. However, in most of these cases serum transaminases and other routine liver function tests will also be abnormal and will point to the presence of liver disease. Patients with cirrhosis and normal transaminases represent a more difficult diagnostic problem, since they may be missed by routine screening tests for liver disease. In patients with cirrhosis of the liver and normal transaminases both fasting and postprandial serum bile acids were abnormal in 93% of the cases, whereas γ -GT (69%), pseudocholinesterase (62%), prothrombin time (62%), γ -globulins (55%), alkaline phosphatase (52%), bilirubin (45%), and albumin (45%) were much less sensitive for the detection of cirrhosis⁵⁵.

Fasting serum bile acids were increased in almost all patients (93%) with primary biliary cirrhosis including the sub-group of patients in whom bilirubin is still at normal serum concentration⁶⁶. Patients with normal bile acid levels had early lesions as judged by histological and

clinical criteria. With progression of the disease total bile acid levels increased and the ratio of serum cholic to chenodeoxycholic acid decreased.

ACUTE AND CHRONIC HEPATITIS

Several studies to evaluate the value of serum bile acids in the discrimination of chronic active and chronic persistent hepatitis were attempted. Marked overlap between the two types of chronic hepatitis was found by several investigators^{3,56,67}. In patients with chronic active hepatitis elevated serum bile acids (measured with a sensitive radioimmunoassay for primary conjugated bile acids) predicted histological evidence of active hepatitis much more reliably than the tests conventionally used for this purpose, such as serum transaminases, bilirubin, alkaline phosphatase and serum proteins⁶⁸. At the time of full remission, serum bile acid levels usually differentiated patients who would remain in remission from those who would subsequently relapse, whereas conventional liver function tests and histological features did not. If elevation of serum bile acids is defined as more than twice the upper limit of normal, elevated levels accurately predicted that relapse would soon follow remission⁶⁸.

GILBERT'S SYNDROME

Serum bile acids are a useful adjunct to the diagnosis of Gilbert's syndrome, ruling out occult structural liver disease and confirming the diagnosis of Gilbert's syndrome in the hyperbilirubinaemic patient. Fasting state levels of serum choly conjugated bile acids are normal in patients with Gilbert's syndrome, even in the sub-group of patients with metabolic abnormalities in sulphobromophthalein and indocyanine green transport⁶⁹⁻⁷¹. Determination of serum choly conjugated bile acids⁷² will add a useful, inexpensive and easily performed test to the evaluation of patients with isolated unconjugated hyperbilirubinaemia. The vast majority of patients with structural liver disease of sufficient severity to produce even minimal hyperbilirubinaemia have elevated serum bile acids^{69,70}. Because of their high extraction efficiency and resultant sensitivity to disturbances in hepatic blood flow and perfusion, normal bile acids are particularly

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reassuring in ruling out occult structural liver disease, such as inactive cirrhosis, a condition which may present as unconjugated hyperbilirubinaemia without other biochemical abnormalities^{55,70}. The bile acid concentrations after an oral loading test with chenodeoxycholic acid did not differ from those found in control subjects⁷³. Recently an abnormal plasma clearance of orally administered ursodeoxycholic acid was reported in Gilbert's syndrome⁷⁴. In this study ursodeoxycholic acid was administered and measured in the unconjugated form. In contrast to cholyl conjugates, which enter hepatocytes largely via the sodium-coupled transport mechanism^{75,76} the hepatic uptake of unconjugated bile acids is less dependent on sodium and may be inhibited by bilirubin⁷⁷. Thus the hepatic uptake mechanism for unconjugated ursodeoxycholic acid differs markedly from that for cholyl or chenyl conjugates⁷⁸.

CYSTIC FIBROSIS

Hepatic involvement is common in cystic fibrosis, and cirrhosis gradually develops in a considerable number of cases. Results of standard liver function tests are generally normal, even though liver biopsy may show rather pronounced changes. Though some patients with cystic fibrosis have elevated serum bile acids^{79,80}, serum bile acid determinations seem to be of poor value in evaluating the extent of liver disease in cystic fibrosis⁷⁹. In some cases there may be decreased intestinal input of bile acids because of intestinal involvement in the disease, and this may invalidate serum bile acid levels as a liver test.

PATENCY OF PORTOSYSTEMIC SHUNTS

The serum bile acid elevations before and after portosystemic shunt surgery can be useful in monitoring patency of a shunt⁷¹, since cirrhotics with portacaval shunts have a significantly higher serum bile acid concentration than cirrhotic patients without portacaval shunt⁸¹.

MONITORING OF BILE ACID TREATMENT

In a previous study Whiting⁸² predicted the composition of bile acids in bile from that in serum. In chenodeoxycholic acid-treated patients with gall stones a close correlation was found between the serum and

biliary proportion of chenodeoxycholic acid. Serum ursodeoxycholic acid also proved to be a very sensitive, specific and convenient means of predicting increased levels of ursodeoxycholic acid in the enterohepatic cycle⁸³. These findings suggest that serum bile acids can substitute for biliary bile acids to test patient compliance in bile acid therapy of gall stones.

BILE ACID MALABSORPTION, STAGNANT LOOP SYNDROME

Patients with bile acid malabsorption due to ileal resection have an altered enterohepatic circulation of bile acids with abnormal diurnal profiles of conjugated cholic and chenodeoxycholic acid in serum^{84,85}. In comparison to healthy subjects, in patients with bile acid malabsorption, normal gall bladder, and normal liver function the postprandial rise of conjugated cholic acid was smaller and decreased with each successive meal⁸⁴, so that the determination of profiles of postprandial conjugated cholic acid levels in serum proved to be a simple and reliable test of bile acid malabsorption⁸⁴.

In patients with stagnant loop syndrome abnormally high values of unconjugated and normal values of conjugated serum bile acids have been observed⁸⁶. This is caused by deconjugation of bile acids by micro-organisms within the lumen of the small intestine. Thus the determination of unconjugated bile acids in serum may be of diagnostic value in patients with stagnant loop syndrome³⁵.

PROGNOSTIC VALUE OF SERUM BILE ACID LEVELS

The prognosis of liver disease can be influenced by many variables, the most important ones being severity and progression of the underlying pathogenic process and functional reserve of the liver. In a prospective study Monroe³ has found that the degree of serum bile acid elevation reflected histological severity in patients with chronic hepatitis. Fasting and postprandial bile acids were more sensitive than standard liver tests in identifying patients with bridging necrosis or cirrhosis. Serum bile acids have been found to correlate closer with quantitative tests of liver function such as the bromosulphophthalein² and indocyanine green clearance tests and the aminopyrin breath test³ than the conventional tests.

Surprisingly little information is available on the utility of serum bile acid levels as a prognostic index of the mortality risk. In patients with stable alcoholic or posthepatic cirrhosis the concentration of bile acids in serum was more closely correlated with the mortality risk than the commonly used clinical and laboratory parameters such as the portal systemic encephalopathy (graded by the number connection test), ascites, albumin, pseudocholinesterase, bilirubin, prothrombin time, and nutritional state. Serum bile acids alone yielded a prediction of mortality comparable to the Child classification⁸⁷. Measured with a commercial radioimmunoassay for primary conjugated bile acids (Becton Dickinson) fasting serum bile acids below 20 $\mu\text{mol/l}$ indicated a good prognosis. Seven per cent of the patients in this group died within 1 year as compared to 6% of the patients with Child grade A. Serum bile acids exceeding 50 $\mu\text{mol/l}$ were associated with a poor prognosis: 67% died within the follow-up period. This outcome was similar to that of patients graded as Child C, of which 70% died within one year⁸⁷. If serum bile acids and Child criteria were combined by means of a logistic regression analysis, the optimal prediction of prognosis could be achieved with the two parameters serum bile acids and number connection test. These results suggest that serum bile acids alone, or in combination with other parameters, may be a clinically useful prognostic index in patients with cirrhosis of the liver.

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3

Advances in Bile Duct Stone Formation and Dissolution*

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INTRODUCTION

Cholelithiasis is one of the most frequent abdominal diseases in North America and Western Europe; 12% of the population are supposed to have gall stones. Most of them are localized in the gall bladder, whereas common bile duct calculi are rare. In autopsy studies cholelithiasis has been found in 6.2–32% of males and in 12.1–57% of females (Table 3.1). The incidence of bile duct calculi varies between 0.5 and 4.5% (Table 3.2).

Choledocholithiasis is a severe disease and complications such as cholangitis, pancreatitis or septicaemia always threaten. Thus a rapid and distinct therapy is required.

This chapter article reviews recent reports on epidemiology and pathogenesis of bile duct stone disease, and deals with one aspect of management, namely dissolution therapy.

EPIDEMIOLOGY OF BILE DUCT STONES

Common bile duct stones originate either from the gall bladder as secondary calculi or are primarily formed in the bile ducts as so-called primary calculi.

* Dedicated to Professor Gerok's 60th birthday

BILE DUCT STONE FORMATION AND DISSOLUTION

Table 3.1 Frequency of cholelithiasis in necropsy series

<i>Author (Ref.)</i>	<i>Region</i>	<i>Males (%)</i>	<i>Females (%)</i>
Zahor <i>et al.</i> ¹⁰⁰	Malmö	29	52
	Prague	32	52
Torvik and Höivik ²	Oslo	13.5	28.6
Bateson and Bouchier ³	Dundee	10.4	23.5
Bainton <i>et al.</i> ⁴	South Wales	6.2	12.1
Lindström ⁵	Malmö	31.9	57
Massarat <i>et al.</i> ⁶	Marburg	22	46
Goebell <i>et al.</i> ⁷	Essen	11.1	26.7

Table 3.2 Incidence of choledocholithiasis in three representative autopsy studies

<i>Author (Ref.)</i>	<i>Region</i>	<i>No. of autopsies</i>	<i>Cases with choledocholithiasis</i>	<i>Percentage</i>
Kozoll <i>et al.</i> ⁸	Chicago	29 618	146	0.5
Zschoch ⁹	Leipzig	34 016	631	1.85
Lindström ⁵	Malmö	2 218	100	4.5
Total		65 852	877	1.3

Secondary bile duct stones

Most common bile duct stones are initially formed in the gall bladder. Thereafter they migrate under unknown conditions to the ductal system. This is supported by some observations: about 15% of patients submitted for cholecystectomy simultaneously have bile duct calculi; this is demonstrated in Table 3.3. Bile duct calculi resemble in morphology and chemistry those from the gall bladder^{1,17,18}.

Simultaneous bile duct stones can be overlooked during surgery. However modern techniques, such as the routine use of intraoperative cholangiography or fiberoptic choledochoscopy, have almost eliminated the problem of undetected retained calculi. Table 3.4 shows that with the improvement of techniques the percentage of retained stones decreased.

Most of the secondary calculi are cholesterol or mixed stones, which are composed of more than 70% of cholesterol^{18,26}. Pigment stones containing less than 25% cholesterol¹⁷ are rare in western countries²⁷.

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Table 3.3 Incidence of choledocholithiasis at time of cholecystectomy

<i>Author (Ref.)</i>	<i>No. of operations*</i>	<i>Incidence of choledocholithiasis</i>	<i>Percentage</i>
Glenn and Beil ¹⁰	5797	586	10
Colcock and Perry ¹¹	1754	139	8
Appleman <i>et al.</i> ¹²	4948	480	9.7
Wenckert and Robertson ¹³	846	160	19
Moller and Santavirta ¹⁴	1937	250	13
Bartlett ¹⁵	4763	744	15.6
Way <i>et al.</i> ¹⁶	952	137	14.4

* Mainly cholecystectomies; in some cases cholecystostomy

Table 3.4 Incidence of retained bile duct calculi after choledocholithotomy

<i>Author (Ref.)</i>	<i>Year</i>	<i>No. of choledocholithotomies</i>	<i>No. of patients with retained calculi</i>	<i>Previous diagnostic procedure</i>
Hicken and McAllister ¹⁹	1964	486	92 (19%)	No operative cholangiography
Hight <i>et al.</i> ²⁰	1959	77	7 (9%)	Operative cholangiography
Hicken and McAllister ¹⁹	1964	400	44 (11%)	Operative cholangiography
Hicken and McAllister ¹⁹	1964	407	16 (4%)	Repeated operative cholangiography
Fogarty <i>et al.</i> ²¹	1968	84	7 (8.3%)	Operative cholangiography
Shore and Shore ²²	1970	100	4 (4%)	Choledochoscopy + operative cholangiography
Longland ²³	1973	37	2 (5.4%)	Choledochoscopy
Leslie ²⁴	1974	91	0	Choledochoscopy
Finnis and Rowntree ²⁵	1977	81	0	Choledochoscopy

However in Japan the proportion of patients with pigment stones approaches 70% in rural localities²⁸ but is decreasing in urban areas where cholesterol stones predominate²⁹.

Primary bile duct stones

Primary choledocholithiasis is uncommon in western areas. Little, and controversial, information is given about frequencies. Data on incidence of gall bladder stones and simultaneous primary bile duct stones are virtually unavailable. In cholecystectomized patients, values vary from 4% up to 60% (Table 3.5).

The discrepancy arises from the uncertainty of determining whether the calculi are really primarily formed in the bile ducts or are secondary in origin.

Madden *et al.*³⁰ gave the extreme value of 60% of patients assumed to have primary bile duct calculi after cholecystectomy, but this very high incidence is based only upon morphological grounds. Braasch *et al.*³¹ aside from a 2-year asymptomatic interval after cholecystectomy, also took the soft brown stones as criteria for primary stones formed in the bile duct. In 55 clinical and autopsy records of patients with congenital absence of the gall bladder and cystic duct, bile duct calculi were found in 20 patients³².

In a carefully performed study Saharia³⁴ established the diagnosis of primary choledocholithiasis by four criteria:

1. the patients had undergone previous cholecystectomy;
2. they had at least a 2-year asymptomatic period following cholecystectomy;
3. stones found in the common bile duct were soft, easily crushable and light brown;
4. there was no evidence of a long cystic duct remnant or biliary strictures resulting from prior surgery.

Only 30 of 758 patients, i.e. 4%, fulfilled these criteria and were identified as patients with true primary common bile duct stones³⁴. Nonetheless it is noteworthy that patients with a gall bladder in place, who develop a primary common duct stone, will be excluded by these criteria. The frequency of common bile duct stones after sphincterotomy is given in a study performed by Seiffert *et al.*³⁵. In a multicentre

Table 3.5 Incidence of primary bile duct calculi

<i>Author (Ref.)</i>	<i>No. of cases</i>	<i>No. of primary common duct stones</i>	<i>Percentage</i>	<i>Classification criteria</i>
Madden ³⁰	126	76	60	Morphological criteria by Ashoff's classification
Braasch <i>et al.</i> ³¹	94	37	39	Previous cholecystectomy; a 2-year (mean 5.8 years) asymptomatic interval; soft brown stones
Gerwig <i>et al.</i> ³²	55	20	36	Congenital absence of gall bladder and cystic duct
Thurston and McDougall ³³	91	9	10	Cholecystectomy; earthy brown stones
Saharia <i>et al.</i> ³⁴	758	30	4	Previous cholecystectomy; a 2-year asymptomatic period; earthy brown stones; no biliary stricture

follow-up trial about 6% of papillotomized patients developed common duct stones within a few years. However it is difficult to determine whether the calculi are primarily formed, since the time interval after papillotomy is not given, and 270 of 954 patients had a gall bladder *in situ* at the time of sphincterotomy.

The composition of common bile duct calculi was analysed in patients undergoing cholecystectomy more than 1 year before, and who had no signs of retained stones postoperatively. In more than 50% of the patients calculi were cholesterol-poor with calcium bicarbonate and fatty acid calcium salts as major components³⁶.

Likewise an Australian group investigated common bile duct stones

of 40 patients whose gall bladder was removed at least 1 year previously¹⁸. Of the 40 patients 25 (62.5%) were found to have cholesterol-type stones and 15 (37.5%) had pigment stones. Patients with pigment common bile duct stones were significantly older than patients with cholesterol common bile duct stones (68 versus 56 years, median) with a greater proportion of males than females (60% versus 28%).

PATHOGENESIS OF BILE DUCT STONES

Most of the simultaneous and retained bile duct stones have cholesterol as a major component. In contrast the recurrent bile duct stones are frequently composed of pigment, especially calcium bilirubinate. The pathogenetic factors which lead to gall stone formation are different for cholesterol and pigment stones respectively.

Bile duct calculi, mainly composed of cholesterol

Investigations into the pathogenesis of cholesterol gall stone disease have been mainly restricted to cholesterol gall bladder stones; but they should also be applicable to cholesterol bile duct calculi, since the latter are considered to be migrated stones.

The formation of cholesterol gall stones depends on three factors: production of lithogenic bile, nucleation and stone growth. Production of cholesterol supersaturated bile is possible by decreased bile acid secretion³⁷, increased cholesterol secretion³⁸ or both the secretion of less bile acid and more cholesterol³⁹. Cholesterol crystallization is a precondition for the formation of gall stones. It has been suggested that the gall bladder adds a mucous glycoprotein as procrystallization factor in patients with gall stone disease⁴⁰. On the other hand Holzbach and co-workers discovered a protein factor, gained from normal bile, which inhibits nucleation⁴¹. The nucleation defect might therefore be due to the presence of a pathological nucleating agent or the lack of a physiological antinucleating agent, or a combination of both.

The mechanism by which cholesterol gall stones enlarge is poorly understood. Crystals have an affinity to each other and stones grow by accretion of cholesterol crystals. Impaired gall bladder motility seems to contribute to stone growth⁴².

Whether these pathogenetic aspects are totally valid for migrated

or retained stones is unknown. Very little information is available on the mechanisms by which cholesterol stones migrate, and how they are modified in composition and size in the common bile duct. Recently a study by Sauerbruch and colleagues revealed that a high rate of presumably migrated calculi had a cholesterol-rich nidus but a cholesterol-poor husk⁴³.

Bile duct calculi, mainly composed of pigment

Pigment gall stones are classified into two main types based on differences of pathogenesis, chemical composition and morphological features. One is the black stone; the other the 'earthy brown' calcium bilirubinate stone.

Black or pure pigment stones are formed in the gall bladder especially in circumstances of metabolic imbalance such as haemolytic anaemia or liver cirrhosis, from where they may migrate to the common bile duct. They are mainly composed of pigment polymer, probably polymerized bilirubin derivatives⁴⁴, calcium phosphate and calcium carbonate⁴⁵.

In contrast to the secondary black pigment stone the calcium bilirubinate stone is the typical recurrent or primary bile duct stone. It is composed of calcium bilirubinate, calcium soaps of fatty acids, small amounts of polymer and a small proportion of cholesterol⁴⁶.

The pathogenesis of pigment stones is still unclear. The major factor is the appearance of an excess concentration of unconjugated bilirubin in bile conditioned by hydrolysis of conjugated bilirubin either by hepatobiliary endogenous, or by bacterial, β -glucuronidase^{47,48}. This is accompanied by increased hydrolysis of lecithin by bacterial phospholipases, which leads to fatty acid calcium soaps⁴⁹.

In normal bile there is no enzyme activity of β -glucuronidase. Two substrates have been suggested to inhibit β -glucuronidase activity: bile acids and glucaric acid. Diet can change the levels of glucaric acid, and possibly contributes to pigment stone formation⁵⁰.

Besides extensive hydrolysis of conjugated bilirubin the relative increase of calcium may lead to supersaturation of bile with calcium bilirubinate. This can be due to a decrease of calcium buffers. Bile salts serve as important calcium binders. In animal models bile salt concentration is decreased in infected bile. Other potential calcium

buffers such as oxalate and citrate might also be metabolized by bacteria⁵¹.

The mechanism for precipitation and enlargement of pigment stones, as well as the polymerization especially of black pigment stones, has been little studied.

Nucleation and accretion of calcium bilirubinate stones may be initiated by binding calcium bilirubinate to a mucoglycoprotein matrix⁵². The condition under which polymerization occurs is unknown. A hypothetical model is that all pigment stones are precipitated as calcium bilirubinate on a mucin glycoprotein matrix. If there is sufficient time, polymerization can occur afterwards and the stone becomes a black pigment stone. If there is bile infection with high β -glucuronidase activity the excess of unconjugated bilirubin leads to rapid precipitation and accretion without having time for polymerization, thus forming calcium bilirubinate stones⁵³.

Clinically, calcium bilirubinate stones frequently have retained non-absorbable suture material at their centre. This was confirmed by Wosiewicz *et al.*³⁶ and Whiting *et al.*¹⁸. They found that in 30% of patients with presumably primary common bile duct stones the most important cause of stone recurrence after surgery was incrustation of unabsorbed suture material. Likewise, especially in patients from the Orient, parasites (i.e. *Ascaris lumbricoides*, *Clonorchis sinensis*) or their ova may serve as a nidus for calcium bilirubinate stones⁵⁴.

The pathogenesis of primary and secondary bile duct stones is shown in Figs 3.1 and 3.2, respectively.

DISSOLUTION OF BILE DUCT STONES

Aside from surgery, stone extraction techniques via T-tube or papilla Vateri have the greatest success in the treatment of common duct stones. However, dissolution has an important role, especially if stone extraction is unsuccessful.

One of the major problems is that most dissolution media are only cholesterol solubilizers so that most of the cholesterol-poor bile duct calculi would be unaffected.

Many solvents have been tried over the years, e.g. ether⁵⁵, chloroform⁵⁶, clofibrate⁵⁷ or heparin^{58,59} but they did not gain widespread use because of doubtful results.

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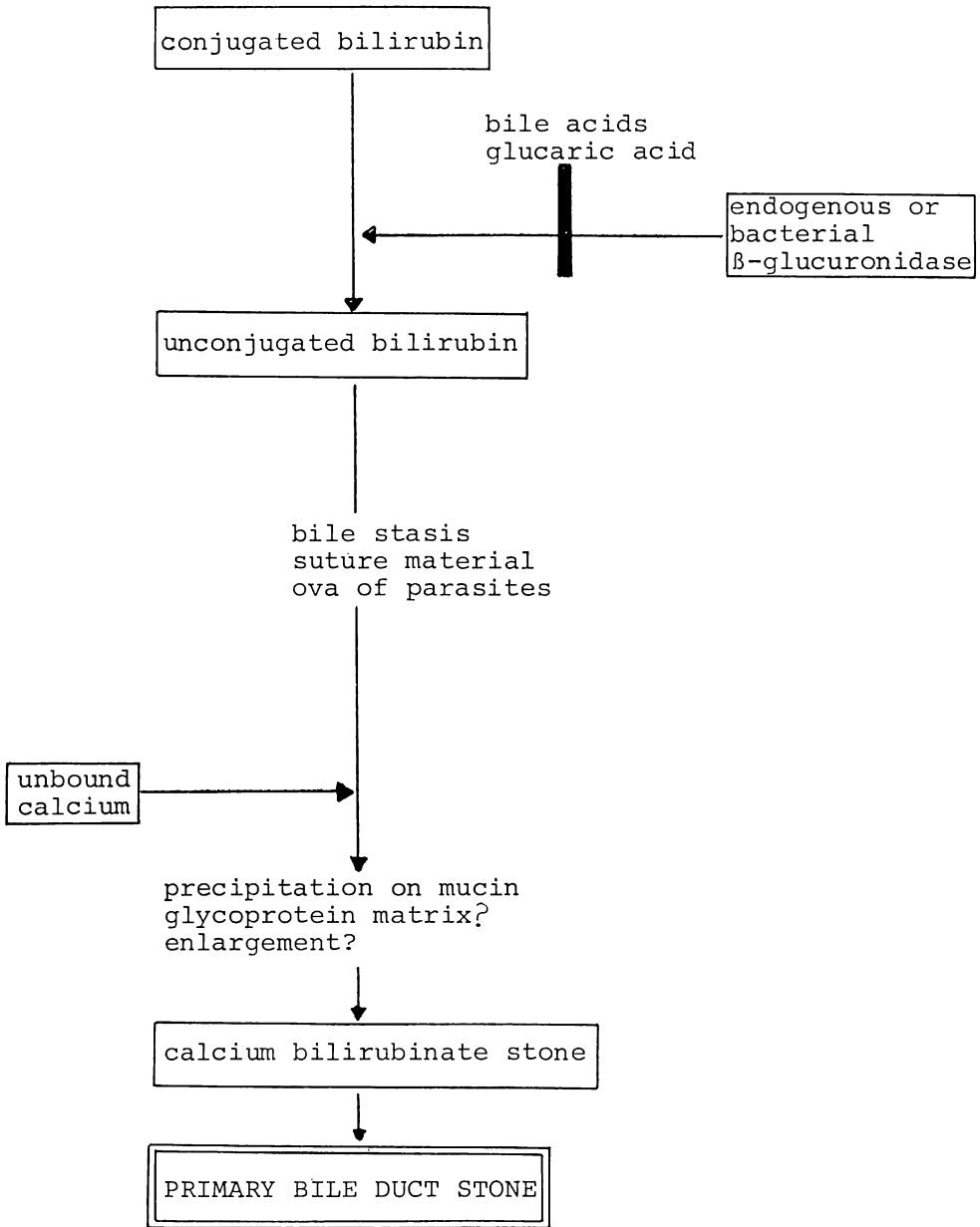


Fig. 3.1 Pathogenesis of Primary Bile Duct Stones

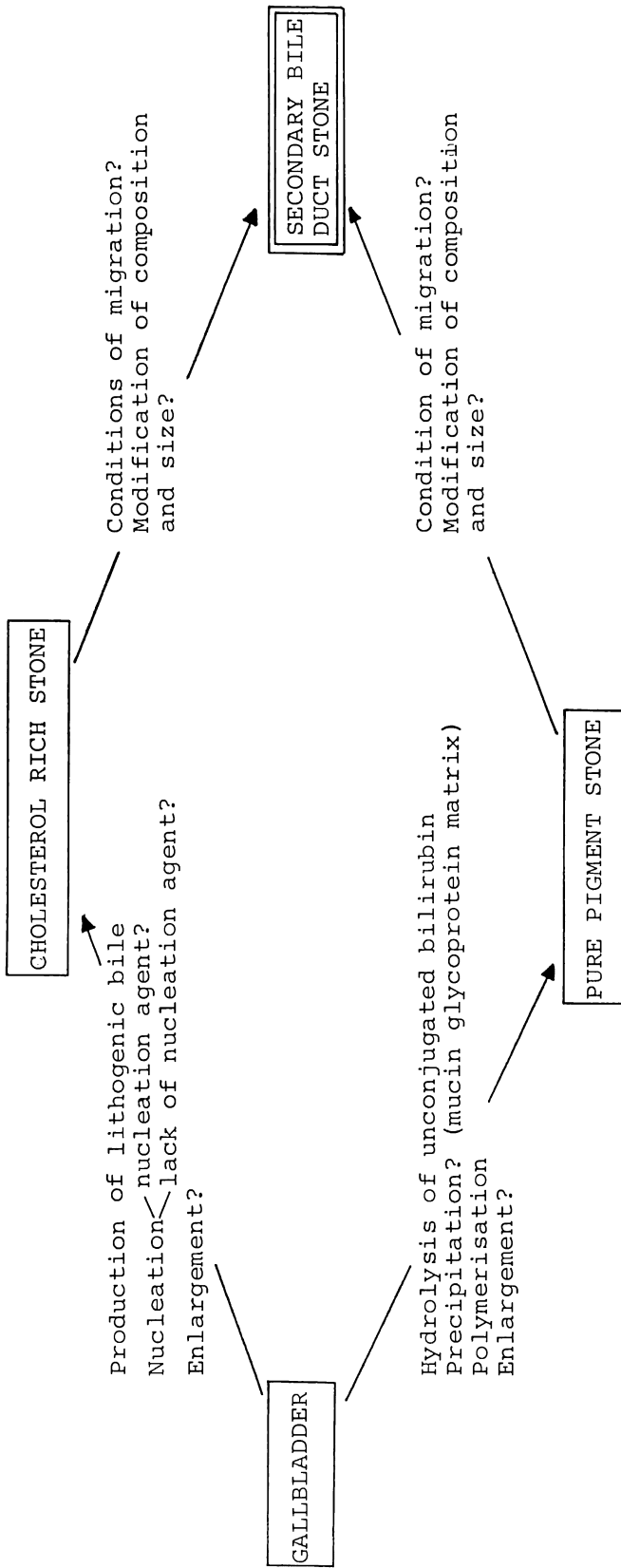


Fig. 3.2 Pathogenesis of Secondary Bile Duct Stones

Dissolution by bile acids

In vitro studies confirm the efficacy of bile salts as cholesterol gall stone solvents⁶⁰.

Cholic acid

Bile salt solutions, particularly sodium cholate, were perfused into the biliary tree to dissolve retained common duct stones. Way and co-workers¹⁶ reported a success rate of 55%. Numerous studies followed, with approximately the same success rate. They are listed in Table 3.6.

Cheno- and ursodeoxycholic acid

Successful oral bile salt therapy for retained common bile duct calculi has been reported with chenodeoxycholic acid (see Table 3.7) and ursodeoxycholic acid^{73,74}. In the study of Salvioli – a double blind randomized trial – only eight of 28 patients completed the investigation. Not surprisingly the others developed complications and left the study because of biliary pain, cholangitis or surgery. Although the authors established feasibility of oral therapy with a success rate of about 50%, other more rapid techniques which are available are preferable.

Dissolution by mono-octanoin

Mono-octanoin, a medium-chain triglyceride, is a potent cholesterol dissolution agent. In *in vitro* studies weight-matched pairs of gall stones were incubated in 150 mmol/l cholate and mono-octanoin solutions respectively. The rate of dissolution was 2.5-fold in mono-octanoin compared with a cholate solution⁷⁵.

Following the *in vitro* studies clinical application was soon reported by Thistle and co-workers. In 12 patients with retained bile duct stones mono-octanoin was infused via T-tube 3–10 ml/h for 4–21 days. In 10 of the 12 patients stone disappearance, or decrease in stone size, occurred⁷⁶. Subsequently other authors confirmed these results; they are listed in Table 3.8. The data reveal that a complete dissolution rate is possible in 55% of all cases, yet 30% do not respond.

In an uncontrolled multicentre trial, which includes in part the

Table 3.6 Dissolution of bile duct calculi by cholic acid

Reference	Year	No. of patients	Applica- tion route	No. of stones	Infusion rate (100 mmol/l NaCA)	Treatment duration (days)	Dissolution rate (in no. of patients) complete partial overall	Percent Side effects
Way <i>et al.</i> ¹⁶	1972	22	T-tube	—	30 ml/h	3-14	—	Diarrhoea
Lansford <i>et al.</i> ⁶¹	1974	6	T-tube	11	30 ml/h	3-10	5	Diarrhoea, pancreatitis
Britton <i>et al.</i> ⁶²	1975	7	T-tube	—	30 ml/h	8-12	4 1 5	Diarrhoea, nausea, abdominal pain
La Russo <i>et al.</i> ⁶³	1975	6	T-tube	—	30 ml/h*	—	—	Diarrhoea, cholangitis
Iser <i>et al.</i> ⁶⁴	1976	14	T-tube	—	30 ml/h	5-14	—	Biliary colic, diarrhoea, dehydration
Christiansen <i>et al.</i> ⁶⁵	1978	7	T-tube	—	†	7-10	—	Nausea, diarrhoea
Sohrabi <i>et al.</i> ⁶⁶	1979	8	T-tube	13	30 ml/h	3-30	—	Nausea, vomiting, abdominal pain
Motson <i>et al.</i> ⁶⁷	1981	9	T-tube	—	—	—	—	†
Total		79					5 43	

* 75-150 mmol/l Na cholate

† Cholate/heparin solution (exact infusion rate not given)

‡ Not given

Table 3.7 Dissolution of non-obstructive bile duct calculi by chenodeoxycholic acid

Reference	Year	No. of patients	Application route	Daily dose	Treatment duration (months)	Dissolution rate (in no. of patients) complete partial overall
Guillet <i>et al.</i> ⁶⁸	1976	1	oral	750 mg	12	1
Barbara ⁶⁹	1976	8	oral	2-15 mg/kg	15	3 2
Thistle <i>et al.</i> ⁷⁰	1978	10	oral	15 mg/kg	>6	— 5
Sonnenshein <i>et al.</i> ⁷¹	1980	1	oral	1 g	12	— 1
Sue <i>et al.</i> ⁷²	1981	8	oral	750 mg	4-8	— 3

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Table 3.8 Dissolution of bile duct calculi by mono-octanoic

<i>Reference</i>	<i>Year</i>	<i>No. of patients</i>	<i>Appl. route (no. of patients)</i>	<i>No. of stones</i>	<i>Stone size (mm)</i>	<i>Infusion rate (ml/h)</i>	<i>Treatment duration (days)</i>
Thistle <i>et al.</i> ⁷⁶	1980	12	T-tube (12)	58	5 × 5–15 × 30	3–10	4–21
Witzel <i>et al.</i> ⁷⁷	1980	16	T-Tube (6) nasobiliary catheter (10)	—	—	7.5	6–25
Wurbs <i>et al.</i> ⁷⁸	1980	10	nasobiliary tube (10)	—	—	—	—
Schenk <i>et al.</i> ⁷⁹	1980	11	T-tube (6), nasobiliary catheter (5)	15	4 × 4–16 × 18	6	2–10
Jarett <i>et al.</i> ⁸⁰	1981	24	T-tube (24)	48	4–14*	2–4	3–112
Gadacz <i>et al.</i> ⁸¹	1981	8	T-tube (8)	16	5–15†	3–7	4–12
Mack <i>et al.</i> ⁸²	1981	20	T-tube (20)	43	—	3–12	1–11
Uribe <i>et al.</i> ⁸³	1981	12	T-tube (12)	14	8 × 6–20 × 15	2–10	2–10
Dawson <i>et al.</i> ⁸⁴	1982	17	T-tube (12), nasobiliary catheter (5)	—	—	—	—
Venu <i>et al.</i> ⁸⁵	1982	9	Nasobiliary catheter (9)	—	—	3–10	until 15
Steinhagen <i>et al.</i> ⁸⁶	1983	6	T-tube, catheter in CBD or CD, PTC, cholecystostomy (2)	—	5–10†	5–10	2–11
Tritapepe <i>et al.</i> ⁸⁷	1984	16	T-tube (15) PTC (1)	25	5–15*	3–4	2–23
Teplik <i>et al.</i> ⁸⁸	1984	11	T-tube (8), PTC (2) cholecystostomy (1)	38	7–27.5†	5	12
Total		172		257			

Abbreviations: CBD = common bile duct, CD = cystic duct; PTC = percutaneous transhepatic catheterization

BILE DUCT STONE FORMATION AND DISSOLUTION

<i>Dissolution rate (no. of patients)</i>			<i>Percent</i>	<i>Side-effects (no. of patients)</i>	<i>No. of stones treated with success</i>			<i>Percent</i>
<i>Complete</i>	<i>Partial</i>	<i>Overall</i>			<i>Complete</i>	<i>Partial</i>	<i>Overall</i>	
5	5	10	83.3	Mild anorexia, emesis, nausea, abdominal discomfort (?)	37	3	40	68.9
12	—	12	75	Nausea, epigastric discomfort (4)	—	—	—	—
6	—	6	60	Nausea, diarrhoea (5)	—	—	—	—
3	3	6	54.5	Nausea, diarrhoea (6), abdominal colic (5)	3	3	6	40
15	5	20	83.3	Diarrhoea (1), biliary pain (3)	35	2	37	77.1
5	—	5	62.5	Abdominal cramps, diarrhoea (?)	8	—	8	50
15	—	15	75	Nausea, vomiting, epigastric discomfort, diarrhoea (?)	34	—	34	79
6	1	7	58.3	GOT, AP † (10) Nausea, vomiting, diarrhoea, abdominal pain (5), gastric ulcer (1), GOT † (1)	6	2	8	66.6
9	1	10	58.8	Not mentioned	—	—	—	—
3	4	7	77.8	Nausea, abdominal discomfort (6), diarrhoea (5)	—	—	—	—
2	2	4	66.7	Vomiting, abdominal pain (5) diarrhoea (1)	—	—	—	—
11	3	14	87.5	Nausea (10), anorexia (2), diarrhoea (1), duodenitis (1)	16	7	23	92
3	6	9	81.8	Amylaseaemia (2), AP † (2), GOT (1), bilirubin † (1), epigastric colicky pain (3)	12	16	28	73.7
95	30	125	72	Total	151	33	184	70

* Average stone diameter (range);
† Stone size (range).

patients of Thistle, Mack, Gadacz and Schenk (Table 3.8), 118 patients with retained common or hepatic duct stones were treated with mono-octanoin via T-tube or nasobiliary catheter. Results, though slightly worse, are comparable to those given in Table 3.8. In 45.8% the stones totally disappeared; in 20.3% there was a reduction in number and size; 33.9% of all patients did not respond⁸⁹.

In a very recent and most extensive report held in 1984 at the British Society of Gastroenterology, Palmer and Hofmann presented complete records of 343 patients with retained biliary calculi, who were subjected to mono-octanoin infusion therapy by different infusion techniques. These were application of mono-octanoin via T-tube (230 cases), nasobiliary catheter (82 patients), PTC (23 patients) or cholecystostomy (8 patients). In 26% stones completely disappeared, in 21% they became smaller, in 9% it was believed that mono-octanoin therapy contributed to successful endoscopic therapy; 33% did not respond to therapy and in 9% treatment had to be stopped because of severe side-effects.

In 67% of all cases side-effects occurred such as abdominal pain (36%), nausea (28%), diarrhoea (15%) and cholangitis (8%). Severe side-effects attributed to therapy were septicaemia in four cases, gastrointestinal haemorrhage in three and pulmonary oedema in one case⁹⁰.

Comparing this recent most extensive survey with the results of the multicentre trial⁸⁹ and those given in Table 3.8, the efficacy of mono-octanoin seems to be lower than initially presumed. From the viewpoint of success, including 'partial success', a rate of 50% may be reached.

Dissolution by GMOC-EDTA-BA (glyceryl-mono-octanoate, EDTA, bile acids)

Based upon these experiences, and especially with respect to the composition of recurrent bile duct stones whose major ingredients are other than cholesterol in 30-50%, Dr Leuschner and co-workers developed a multicomponent solution consisting of bile acids, disodium-EDTA, glyceryl-mono-octanoate and carnosin as buffer⁹¹. The rationale for the use of this mixture is that the EDTA solution will chelate calcium salts such as calcium bilirubinate and calcium palmi-

tate, and that mono-octanoin and bile acids will solubilize cholesterol. Carnosin is used to buffer the solution to alkaline pH in order to improve the chelating effect of EDTA.

These workers found that the mixture could completely disintegrate a brown pigment gall stone *in vitro* after 36 h of incubation⁹². In a first clinical trial using alternating infusions of mono-octanoin and bile salt-EDTA through a nasobiliary catheter, they achieved a partial or complete dissolution of bile duct stones in 12 of 20 patients⁹¹.

In a second study following the same procedure they had partial or complete success in six of 10 patients⁹³ and in a third study in 57% of the patients studied⁹⁴. However, since the composition of the gall stones was not ascertained, the interpretation of the role, especially of EDTA, is difficult. Considering the clinical success rates it needs to be proven whether the combined solution offers any advantage over mono-octanoin. Moreover, the *in vitro* experiments of Allen and co-workers⁹⁵ in 1983 seem to cast doubt on the superiority of GMOC-BA-EDTA. This group compared glyceryl-mono-octanoin (GMOC), alternating GMOC/bile acid-EDTA solution, unmodified mono-octanoin and mono-octanoin diluted in 10% of water, and its capacity to dissolve gall stones of similar size containing 94% and 40% cholesterol respectively. GMOC offered no advantage over mono-octanoin and alternating GMOC/BA-EDTA was inferior to mono-octanoin alone for both cholesterol and mixed stones⁹⁵.

Altogether controversial observations exist, and a conclusive answer is lacking since a controlled clinical trial comparing the efficacy of the different irrigation media has not been performed to date.

Dissolution by MTBE (methyl-tert-butyl ether)

In 1983, in a first report in abstract form, methyl-tert-butyl ether (MTBE) was introduced as a rapid cholesterol gall stone solubilizer *in vitro* and in animal studies⁹⁶. In additional experiments the authors confirmed that MTBE dissolves human cholesterol stones 50 times faster than mono-octanoin. In dogs MTBE dissolved 14 of 15 surgically implanted human cholesterol stones within 4-16 h. No effect was observed upon a pure pigment stone. Minor side-effects such as nausea, hypersalivation and vomiting occurred in two-thirds of the dogs investigated⁹⁷.

In a first clinical approach the same group achieved in one patient dissolution of an intrahepatic stone via nasobiliary catheter within 4 h, and in two further patients gall bladder stones were dissolved via transhepatic catheter placement within 7 h. The agent was well tolerated without major side-effects⁹⁸.

In a second clinical study performed by an Italian group MTBE was administered via T-tube within 3–8 h in three patients with retained non-obstructive common bile duct stones. MTBE failed to dissolve the calculi, whereas the subsequently instilled mono-octanoin solution induced partial dissolution in one patient and complete dissolution in the other two patients within 21–29 days⁹⁹.

Side-effects of MTBE were nausea, somnolence and histological evidence of duodenitis. The authors conclude that MTBE is only efficient in closed environments (such as the gall bladder), where calculi are continuously contacted by the dissolution agent. In fact, studies in a gall bladder and bile duct model showed that stone–solvent contact is a very important factor in achieving an acceptable rate of gall stone dissolution. Because of their physical properties mono-octanoin as well as MTBE float on bile, whereas gall stones sink. Mono-octanoin, but not MTBE, stirred with bile induce sufficient solvent–stone contact and increase dissolution rate. MTBE is most effective if it is excluded from bile and simultaneously stirred¹⁰¹.

Clearly further experiences are needed to establish the efficacy of MTBE in gall stone disease.

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4

Lasers in Gastroenterology

G. Lux and Ch. Ell

The principle of light amplification by stimulated emission of radiation (laser) has been known for the past 25 years. In 1960 Theodor Maiman succeeded, with the aid of a ruby laser, in producing monochromatic and coherent electro-magnetic radiation¹.

The development of efficient, flexible light-transmitting fibres along which laser light can be conducted into the hollow cavities of the gastrointestinal tract, without any appreciable transmission losses, made possible the application of the laser in gastroenterological endoscopy.

The methodic development of the 'laser in gastroenterology' was promoted by Silverstein, Washington; Dwyers, Los Angeles; Kiefhaber, Munich; Frühmorgen, Erlangen. On 5 June, 1975 Frühmorgen in Erlangen succeeded in carrying out the first successful endoscopic laser photocoagulation in the gastro-intestinal tract².

GENERAL PRINCIPLES OF THE LASER

Laser light is characterized by its monochromaticity, coherence, and high parallelism. These characteristics are achieved through the principle of induced emission of radiation: when electromagnetic radiation (photons) impinges upon excited electrons, the latter are induced to give up photons, the wavelength and phase of which correspond exactly with those of the impinging radiation. In this manner light amplification of the primary photon wave, characterized by mono-

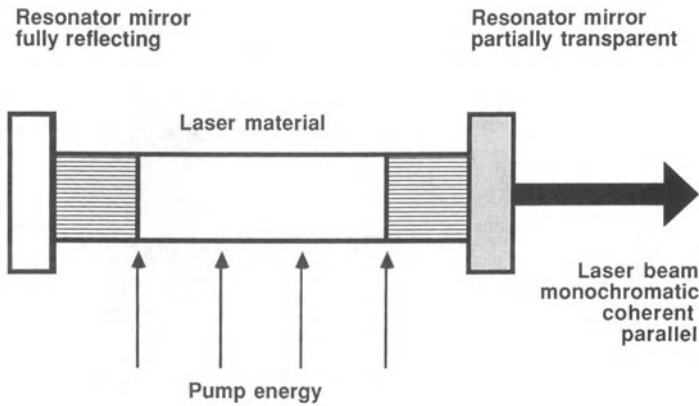


Fig. 4.1 The principle of a laser resonator

chromaticity and coherence, is achieved. The principle of light amplification by the induced emission of radiation may be explained in the case of the neodymium–YAG laser – a so-called solid state laser – which can serve as an example (Fig. 4.1). Neodymium atoms, which are to be excited for subsequent photoemission, are contained within an yttrium–aluminium garnet crystal. This latter, the so-called laser rod, is arranged along the longitudinal axis of an elliptical resonator. In order to excite the active laser medium, energy must be applied from outside – so-called optical pumping. In the case of the neodymium–YAG laser this is achieved with the aid of high-power-density krypton or iodine–tungsten lamps. On plane-parallel sides of the resonator housing at each end of the laser rod, mirrors are arranged, one reflecting almost completely (99% reflectivity), the other being semi-transparent. Optical pumping stimulates the electrons of some of the atoms to move up to a higher energy level. Some electrons drop back spontaneously into the ground state, emitting radiation while doing so – part of which is lost in the form of lateral scattering. Some of the photons are emitted along the axis of the laser rod, and reflected by one of the two mirrors; on their return journey they are either absorbed or cause excited atoms to emit a photon. When, on passage of the light wave through the laser medium, more than 50% of the neodymium atoms are in an excited state, more photons are liberated by stimulated emission than are absorbed by non-excited atoms.

With a suitable distance between the mirrors the continued reflection of the light between them leads to the production of a stationary wave which, as a result of the constant supply of energy through pumping represents an additional increase in light amplification. If required, a given portion of the laser light can be 'coupled out', focused by means of a system of lenses, and then transmitted via a flexible quartz fibre to the site of application^{3,4}.

LASER SYSTEMS

In accordance with the physical state of the active laser medium, a differentiation is made between solid-state lasers (e.g. neodymium-YAG laser, ruby laser), liquid lasers (e.g. dye lasers), and gas lasers (e.g. argon laser, CO₂ laser).

The laser systems presently used in medicine are designed predominantly for continuous operation. The power output delivered is between 10⁻³ and 10² watts.

An increase in power output over a short period can be achieved by pulsed laser operation. The simplest method for producing short light pulses of high power density is to pulse the excitation energy of the laser (optical pumping). In this manner, power outputs of 10² to 10⁵ watts can be achieved for periods of 10⁻² to 10⁻⁶ seconds (Fig. 4.1).

A further principle, the Q-switched laser, is as follows: with continuous optical pumping, maximum excitation energy is stored within the laser crystal, and is emitted in the form of a very short high-intensity pulse of light. Q-switched laser systems are capable of producing output powers of 10⁶ watts, at a pulse duration of up to 10⁻⁹ seconds (for further details see Refs 5 and 6).

LASER-INDUCED PHOTOPHYSICAL PROCESSES

The nature and extent of photophysical processes induced by laser light are determined by the following parameters:

1. the physical parameters of the radiation (wavelength, exposure time, output and energy density);
2. optical parameters (reflection, scattering, transmission and absorption);

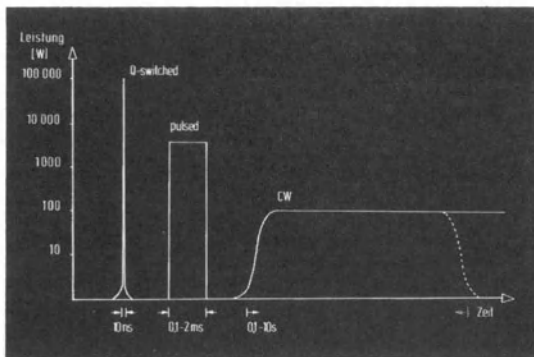


Fig. 4.2 Continuous-wave laser and pulsed laser: principle of radiation; y-axis: energy, x-axis: time

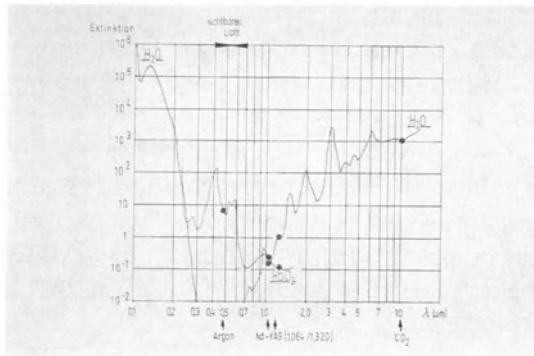


Fig. 4.3 Extinction rate of H₂O, hemoglobin and melanin; ↑argon-laser; ↑↑Nd:YAG laser

3. thermal parameters (thermal conductivity, specific heat and the local diffusion rate of the tissue).

Four major physical processes can be differentiated⁷. Most commonly known is the *thermal effect* of laser light (Fig. 4.2). Irreversible tissue damage with denaturation of protein occurs at temperatures of about 60 °C. Between 90 °C and 100 °C tissue water begins to boil. At higher temperatures charring takes place. The black carbon on the tissue surface increases absorption; if the application of laser energy is continued the temperature can rapidly rise to several hundred degrees, or even exceed 1000 °C, resulting in melting, burning and, finally vaporization of all chemical compounds⁸.

Chemical processes are induced at extremely low energy densities and relatively long exposure times. By combining photosensitizing substances and the appropriate laser light, cytotoxic processes can be induced selectively^{9,10}. The third photophysical principle is the *conversion of light energy into mechanical energy* (electromechanical effect). The optical breakdown required for this is characterized by the stages of plasma formation, plasma expansion, and the development of a shock wave. These effects can be achieved with Q-switched laser systems only¹¹, providing extremely high energy densities at the focus of the laser. So far, little is known about the significance of the so-called *photoablative laser effect*. Excimer lasers operating in the ultraviolet range can produce energy densities between those of Q-switched and those of the continuous-wave lasers by pulsed mode. With exposure times in the nanosecond range, non-thermal tissue-destroying or tissue-ablating effects can be achieved through the selective breakdown of intramolecular bonds (photodissociation)^{12,13}.

LASER SYSTEMS IN GASTROENTEROLOGY

At the present time the system preferred by most gastroenterologists is the neodymium–YAG laser. Today, argon and carbon dioxide lasers are of little importance. If the absorption spectra of haemoglobin (blood) and water (water-containing tissue), are compared, the different effects of the various laser systems can be understood (Table 4.1; Fig. 4.3). With its wavelength of $478 \mu\text{m}$ – close to the absorption maximum of haemoglobin – the argon laser is highly suitable for haemostasis and coagulation. At a wavelength of $10\,600 \text{ nm}$ – the CO_2 laser – maximum absorption of light occurs in water, causing vaporization of the water content and disruption of the tissue. Utilizing this principle the carbon dioxide laser may be called a ‘laser scalpel’¹⁴. Both argon and carbon dioxide laser light is primarily absorbed at the surface of the tissue with a low depth of penetration. In contrast to these two laser systems, the depth of penetration of the neodymium–YAG laser is relatively high, since the absorption of light by organic material in the near-infrared range at 1064 nm is small, while transmission in water is high. For this reason the optical parameter of light scattering is of importance here. At low output powers (30–40 watts), a homogeneous distribution of the radiation within the tissue occurs,

Table 4.1 Relationship between temperature and tissue event (according to Ref. 33)

<i>Critical temperature (°C)</i>	<i>Histological event</i>	<i>Endoscopic manifestation</i>
45	Cell death, oedema endothelial damage, vasodilatation	Erythema, oedema cuff
60	Protein coagulates	Tissue turns grey-brown, blood turns black
80	Denatured collagen contracts, blood vessels constrict	Tissue 'puckers'
100	Tissue water boils	Vaporization causes a divot
210+	Dehydrated tissue burns	Blackened tissue disappears ± glowing embers

causing homogeneous coagulation necrosis. At higher powers (80–100 watts), vaporization effects are obtained^{7,15}. Argon and pumped dye lasers with a wavelength of 630 nm are already being used for photodynamic therapy^{10,16}. The utilization of the phenomenon of autofluorescence of photosensitized tumours exposed to a wavelength of 407 nm (krypton laser) makes selective tumour diagnosis possible, at least experimentally. However, there are problems such as making the autofluorescence visible, and there is limited documentation on the tumour-destroying effect and tumour cell selectivity. The future role of laser diagnosis and therapy with photosensitizing substances still needs evaluation in the field of gastroenterology. Furthermore the photosensitizing substances presently available are associated with major systemic side-effects¹⁷.

At the present time basic experiments are necessary to evaluate the possible role of different kinds of laser systems: neodymium-YAG laser systems with a wavelength of 1320 nm for tumour therapy, pulsed neodymium-YAG and pulsed dye lasers for lithotripsy and treatment of oesophageal varices, and the so-called excimer laser for tumour destruction and/or lithotripsy.

ENERGY TRANSMISSION – ENERGY APPLICATION

In all laser systems employed in gastroenterology the transmission of energy is effected through a flexible quartz fibre¹⁸ enclosed within a Teflon sheath to protect against fracture. The neodymium–YAG laser (wavelength 1064 nm), is usually transmitted through a fibre with a diameter of 0.6 mm. The tip of the fibre is bathed in a constant flow of carbon dioxide to prevent its contamination with fluids and necrotic tissue, and thus its self-destruction by burning.

For the application of light energy the non-contact method has proved of value. The distance between the tip of the fibre and the tissue to be treated is 3–10 mm. A pilot light (helium–neon laser) is employed to facilitate aiming⁸.

Recently the contact method of laser treatment using sapphire tips has been recommended. An advantage of the contact method might be that less energy is needed to achieve identical effects, i.e. one-third to one-quarter of the energy has to be applied due to the higher energy density and the smaller loss of energy by backscattering. In the case of the non-contact method the loss of energy can be as much as 40%. Using sapphire tips, the tissue-damaging effect can be accurately calculated and estimated. With the non-contact method the distance between the tip of the fibre and the tissue varies, and thus also the energy density effective in the tissue. The use of water irrigation eliminates the need for gas insufflation, which is painful for the patient.

A further variation of the contact method applies a 'naked' quartz fibre without insulation at the distal tip directly to the tissue. A continuous flow of gas can be avoided, and destruction of the tip of the fibre is accepted²⁰.

At the present time there are only a few applications for the contact method, such as total tumour stenosis, over-growth of an endoprosthesis with tumour tissue, and high-grade stenoses in the large bowel.

ABLATIVE LASER THERAPY

One prerequisite for the development of operative endoscopy was cutting and coagulating by high-frequency diathermy. In principle, high-frequency diathermy can be replaced in all its fields of application

by the laser beam. The question must be asked, however, whether this will result in advantages or disadvantages in respect to diagnosis, complications or economic aspects.

The ability of the laser to vaporize or coagulate tissue opens up the possibility of removing, either completely or partially, benign or malignant neoplasias within the gastro-intestinal tract. In addition to these theoretical considerations, ablative laser therapy is also based on practical experience with laser treatment of bleeding tumours and of tumour stenoses, since here, apart from the desired effect of haemostasis, the removal of neoplastic tissue and/or the re-canalization of tumour stenoses proved feasible. The first attempts at treating gastrointestinal tumours and tumour stenoses with the laser²¹⁻²³ were made long after it had been demonstrated that the laser was capable of ablating tumour tissue²⁹. Subsequently, further results of palliative laser therapy in tumours of the upper gastrointestinal tract were published^{19-23, 25-41}.

Palliative tumour therapy

Tumour stenoses of the upper gastrointestinal tract

By the time the diagnosis is established, slightly less than one-half of the patients with oesophageal carcinoma are no longer operable⁴². Similar figures apply for carcinoma of the cardiac region.

The cardinal symptom of tumour stenosis of the upper digestive tract is dysphagia with the sequential loss of weight, malnutrition, aspiration and, not infrequently, social isolation, often associated with frequent periods of hospitalization. Thus the objective of palliative therapy is the 'normalization of the patient's life' by enabling him to swallow food and saliva. Bleeding from tumours of the upper gastrointestinal tract is far less frequently seen. An increase in the survival rate is not to be expected from local measures such as laser coagulation.

Applications for endoscopic laser palliation are tumour stenoses of the upper gastrointestinal (GI) tract caused mainly by oesophageal and gastric carcinoma, and anastomotic recurrences after resection of oesophageal or gastric tumours. A prerequisite for palliative laser therapy is local or general inoperability, which has to be established

unequivocally by preoperative procedures; otherwise explorative surgery has to be performed. In the case of bleeding from tumours of the upper GI tract, laser haemostasis is also employed in the case of operable patients.

Laser therapy is not applicable to oesophago-bronchial or oesophago-mediastinal fistulae.

Materials and method As already mentioned, the powerful neodymium–YAG laser (wavelength 1064 nm), which is suitable both for coagulation and vaporization of tumour tissue, has become generally accepted. The power output required to treat tumours in the upper gastrointestinal tract is between 80 and 100 watts. Individual reports on tumour destruction via coagulation necrosis induced by an argon laser are mainly of historical interest^{43,44}. Photodynamic treatment with the aid of argon-pumped dye lasers and hematoporphyrin derivatives (HpD) is still in its early stages, and this form of treatment is more likely to be suitable for the curative therapy of small malignancies rather than for palliative tumour treatment^{10,45}.

Contact-free application of laser energy is generally accepted. Only when inflammable material such as bridging tubes might be exposed to the laser beam is the contact method to be recommended. Most authors prefer the flexible quartz fibre as transmission system (MBB, Munich). The fibre is kept clear of blood and necrotic tissue by a constant flow of CO₂. The divergence angle of 10° of the emitted laser beam permits the distance between the tumour and the tip of the fibre to be varied between 3 and 10 mm. The three-channel endoscope (TGF1DL Olympus, Tokyo) with an integrated triconical quartz fibre (divergence angle 4.2°) as originally described by Nath¹⁸, is less frequently used. The latter system does not need a constant flow of gas.

Most authors prefer to use routine gastroscopes, for example GIFQ₁₀ or GFXQ₁₀ (Olympus, Tokyo), while others prefer oblique optics (GIFK₁₀, Olympus, Tokyo) or those with two working channels. Since laser energy reflected by the tissue can damage the tip of the instrument, endoscopes with laser-resistant tips should be used. The latter are of white colour and of various materials (Teflon, ceramic).

The first reports on palliative laser therapy^{21–23,31,32,41}, recommended starting at the proximal end of the tumour (Fig. 4.4). The laser beam is guided concentrically around the luminal opening while widening

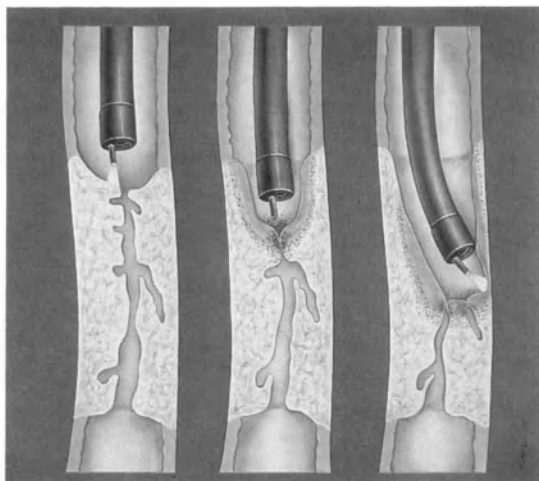


Fig. 4.4 Laser therapy beginning at the proximal margin with stepwise progression to the distal parts, in the middle part correct axis, in the right part 'via falsa' with danger of perforation

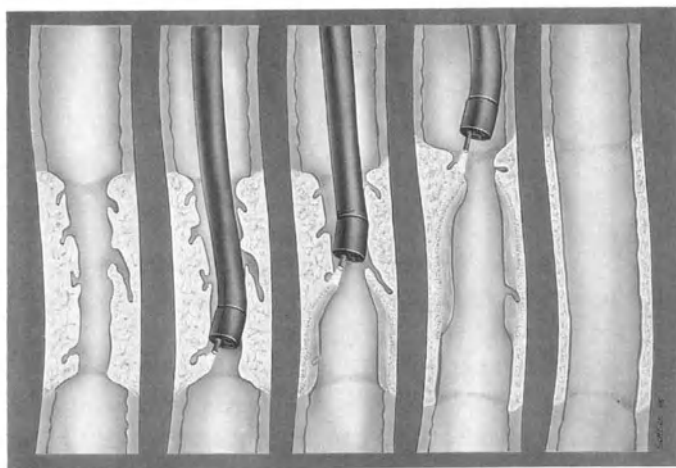


Fig. 4.5 Tumour stenosis passable to the endoscope: beginning of laser therapy working in oral direction

the circles described by the laser beam. The intention is to vaporize the tissue of the central areas and to cause white necroses towards the periphery. The procedure is repeated every other day. After this interval laser oedema and necrotic tissue disappear in most cases.

The power output selected is 90–100 watts with a pulse duration of 2 s or more at a distance of 3–10 mm. Laser coagulation beginning at the proximal end of the tumour stenosis is associated with an increased risk of perforation. The complication rate has been reported to be between 12 and 30%^{34, 37, 46}.

In our experience, initiating laser application at the distal end and working oral-ward is much easier, and there is less danger of perforation (Figs. 4.5, 4.6). The big advantage of this technique is that the direction of the intestinal axis is permanently visualized. Dilatation of the tumour stenosis is a necessary additional procedure prior to coagulation.

A third possibility is application of the laser along a laser-resistant guide probe as described by Ell⁴⁷ (Fig. 4.7). The guide probe is passed through the stenosis under endoscopic or fluoroscopic control. Coagulation is begun at the proximal margin of the tumour and the laser works around the laser-resistant guide probe in the distal direction, until the lumen is visualized or the stenosis can be negotiated with the endoscope. Integrated within the guide probe is an aspiration channel, through which smoke and CO₂ gas can be suctioned off.

Results Between 1 June 1984 and 31 August 1985, 62 patients with malignant stenoses of the uppergastrointestinal tract were submitted to laser therapy at the Medical Department of the University Hospital Erlangen. Twenty-three of the patients (37.1%) were curatively inoperable, 39 (62.9%) were inoperable for general reasons such as age or additional serious illness. Table 4.2 shows the location, degree and length of stenoses.

In the case of a tumour stenosis permitting passage of the endoscope, laser therapy was employed as the sole method, otherwise a combination of dilatation and laser therapy, or laser therapy along a laser-resistant guide probe, was used. On average, three laser sessions (range 2–6) and two dilatation sessions (range 1–5) proved necessary. The time interval between the treatment steps was usually 1 day.

An average of 3200 joules (range 1000–10 000) of energy were

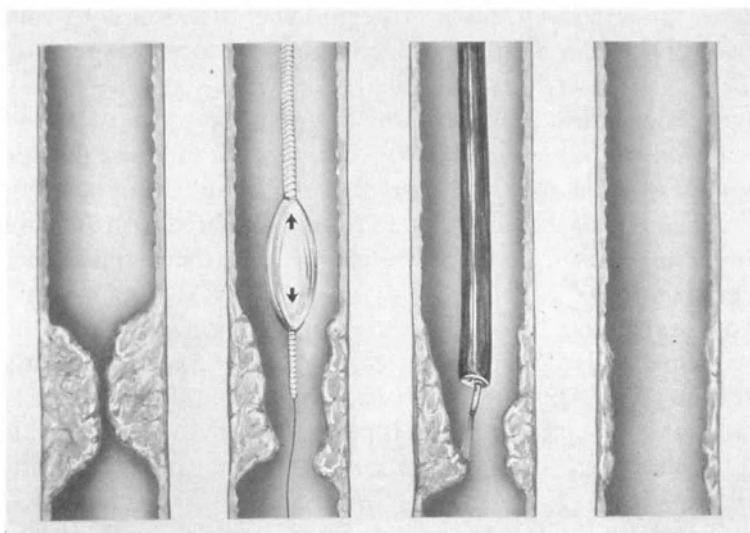


Fig. 4.6 Tumour stenosis requiring combined therapy by dilatation (Eder-Puestow dilator) and laser coagulation, working from distal to proximal margin

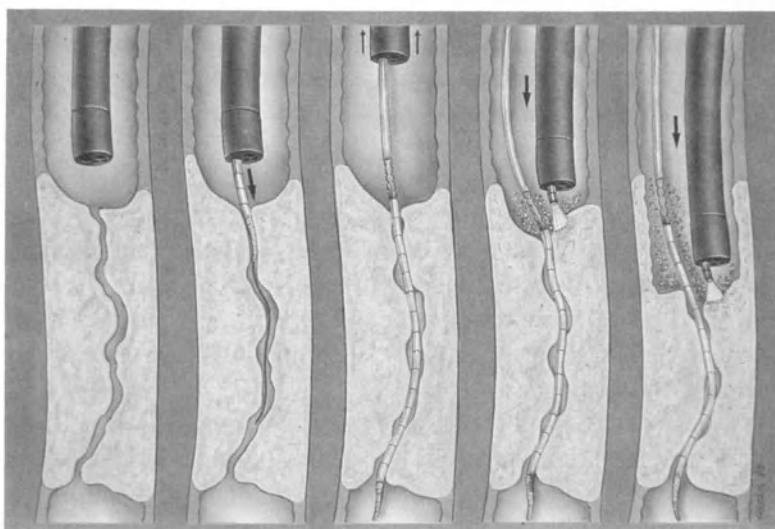


Fig. 4.7 Laser therapy carried out along the laser-resistant guide probe from proximal to distal in a filiform stenosis; the correct direction is guided by the probe

Table 4.2 Tumour characteristics prior to laser therapy ($n=62$), Medical Department, University Hospital, Erlangen

<i>Localization</i>		<i>Degree of stenosis</i>	
Oesophagus	39	Stenosis endoscopically	37 (59.6%)
proximal	5	impassable	
middle	18	(lumen < 9 mm)	
distal	16		
Stomach (cardia)	15	length of stenosis	
Anastomosis		> 4 cm	32 (51.6%)

Table 4.3 Results of palliative laser therapy ($n=62$), Medical Department, University Hospital, Erlangen

Initial treatment successful (solid food can be eaten)	50/62 patients (80.6%)
Complications (total)	3/62 patients (4.8%)
Perforation	2/62 patients
Esophago-mediastinal fistula	1/62 patients
Mortality	1/62 patients (1.6%)
Dysphagia-free interval	
3–6 weeks	29/42* patients (69.1%)
> 3 months	9/42* patients (21.4%)

* 20 patients were not followed up

employed per session. The total energy applied during initial treatment varied considerably, depending upon the tumour on the average 8900 joules (range 4800–63 000) were applied.

At the end of treatment, four-fifths of the patients were able to eat solid food again (Table 4.3). In 12 patients the objective of treatment was not achieved. Of these, three patients had stenoses with invasion of the larynx and neurological problems in swallowing. Four patients were able to take only liquids after treatment, although the endoscope could readily be passed through the extended stenosis. One patient was in an extremely debilitated condition; one patient refused further treatment after the first laser session.

In three patients (4.8%) severe complications were observed (Table 4.3). In the first case, during laser therapy the patient who had pre-

viously undergone radiotherapy revealed an oesophago-mediastinal fistula which, in the same session, was closed with a rapidly hardening amino acid solution applied via the endoscope. In a second patient perforation into the abdominal cavity occurred and required surgical repair. In the third case, after bougienage and laser therapy of a bronchial carcinoma, perforation leading to septic mediastinitis and subsequent death occurred.

In somewhat more than two-thirds of the patients who presented themselves for follow-up examination, a clinically relevant recurrent stenosis occurred after 3–6 weeks (Table 4.3). Recurrence-free intervals of more than 3 months were the exception rather than the rule.

The experience of the Erlangen Clinic was confirmed by an inquiry carried out by Ell¹⁹ covering a total of 1359 patients (Tables 4.4–4.6).

Alternatives to laser therapy

Possible non-surgical alternatives to laser coagulation are: long-term bougienage, implantation of ‘bridging’ endo-prostheses; radiotherapy, including after-loading; and chemotherapy with cytotoxic agents: sclero-therapy of tumour stenoses⁴⁸, electroresection⁴⁹, ‘hot bougienage’⁵⁰, percutaneous endoscopically controlled gastrostomy (PEG), or the creation of a Witzel fistula, are rarely employed.

Implantation of intestinal bridging tubes The method of implanting oesophageal endoprotheses, which is largely employed today under combined endoscopic and fluoroscopic control, has been standardized^{51–57}.

The indication for the implantation of an oesophageal ‘bridging tube’ is tumour stenosis in oesophageal, cardiac or gastric carcinoma, bronchial and mediastinal tumours, and oesophago-bronchial fistulae, usually with underlying malignancy. Prerequisites for this procedure are that surgery is not indicated, radiotherapy holds out no chance of success, the tumour is located not less than 2 cm distal to the upper oesophageal sphincter, and the patient has an expected survival time of at least 4–6 weeks and is co-operative, or can be motivated.

Results At the Medical and Surgical Department of the University Hospital at Erlangen the indication for endoscopic implantation of

Table 4.4 International inquiry: patients, prior treatment and pre-therapy tumour characteristics (hospitals with > 15 treated patients. *n* = 20)

Treated patients (total) (<i>n</i>)	1184 (100%)
Localization	
Oesophagus	816 (68.9%)
proximal	174 (14.7%)
middle	348 (29.4%)
distal	294 (24.8%)
Stomach	265 (22.3%)
Anastomosis	100 (8.4%)
Duodenum/Jejunum	3 (0.3%)
Degree of stenosis	
Stenosis endoscopically impassable (lumen < 9 mm)	600 (55.7%)
Length of stenosis > 4 cm	686 (57.9%)
Pre-treatment	
Radiotherapy	152 (12.8%)
Chemotherapy	50 (4.2%)

Table 4.5 International inquiry; premedication and technical data on laser therapy (hospitals > 15 treated patients; *n* = 20)

Premedication	
Tranquillizers + analgesics	10/20
Tranquillizers	5/20
Analgesics	1/20
Hypnotics	1/20
Tranquillizers + analgesics/anaesthetics	3/20
Laser sessions (initial treatment)	
on average 3 (2–6)	
Energy emitted	
on average 11 000 (7000–24 000) joules	
Technique employed	
Combined laser therapy (laser + bougienage) and laser therapy alone	16/20
Laser therapy alone	4/20

Table 4.6 International inquiry; results of palliative laser therapy (according to Ref. 19) ($n = 1184$)

Initial treatment successful (solid food can be taken)	983 (83.0%)
Complications (total)	49 (4.1%)
Perforation	25 (2.1%)
Fistulas	9 (0.8%)
Haemorrhage	8 (0.7%)
Sepsis	7 (0.6%)
Complications resulting in death	12 (1.0%)

an endoprosthesis was established in 170 patients, comprising 136 men having an average age of 63.6 and 34 women with an average age of 73.2 years. In 10.9% of the cases the condition was an oesophago-tracheal fistula resulting from such neoplasms as oesophageal or lung cancer or, in one case, underlying tuberculosis. Almost 90% of the oesophageal 'bridging tubes' were implanted for the palliation of tumour stenoses.

Of the 170 tube implantations attempted, 5.8% were unsuccessful, obstruction of the tube occurred in 3.5%, and dislodgement in 7.0%, the initial failure being eliminated endoscopically by reimplantation in 5.8%. Of the 10 perforations, five were associated with the death of the patient.

On average, patients with a 'bridging tube' survived for 8 weeks, with a maximum survival of 60 weeks. In all patients in whom endoscopic implantation was successful, a marked improvement in dysphagia was observed.

Compared with the surgical placement of oesophageal 'bridging tubes', endoscopic endoprosthesis implantation is associated with an appreciably lower complication rate⁵⁸.

Bridging tubes and laser coagulation in tumour stenoses of the upper gastrointestinal tract As a major alternative form of treatment of tumour stenoses of the upper gastrointestinal tract, laser coagulation vies with the placement of an oesophageal 'bridging tube'. Radiotherapy and chemotherapy are usually employed in combination with the re-establishment of luminal patency through the use of the laser, or implantation of an endoprosthesis.

Table 4.7 Tumour stenosis, upper gastrointestinal tract

<i>'Tubes'</i>	<i>versus</i>	<i>Laser</i>	
⊖ Implantation of plastic material	Mode of action	Diminution of tissue	⊕
⊕ 90%	Success rate	90%	⊕
⊖ 2-3(5)%	Lethality	1%	⊕
⊖ Dislocation perforation	Complications	Restenosing	⊖
⊕ Occlusion possible	Fistulae	Occlusion not possible	⊖
⊖ Not possible	Localization		⊕
Not possible	Proximal	Possible	
Not possible	Distal	Possible	
Not possible	Total stenosis	Possible	
⊕	Costs		⊖
⊕	Hospitalization		⊖
⊕ advantage			
⊖ disadvantage			

For this reason we first compare the advantages and disadvantages of tube implantation and laser coagulation (Table 4.7). With either technique the patency of the oesophagus can be maintained in more than 90% of cases^{27, 33-35, 41, 51-57, 59, 60}. The complication rates of the two procedures are only partially comparable, since in the case of perforation, tube implantation is at the same time the treatment of choice. Furthermore, a number of complications associated with tube implantation, for example obstruction or dislocation, can be corrected by a renewed implantation. In many respects that would be comparable to re-stenosis following laser coagulation. With respect to mortality rates, a retrospective comparison can be only approximate, but it does appear that the mortality rate associated with laser coagulation – roughly 1% – is below that observed in tube implantation – approximately 2-3% up to 5% and more.

A disadvantage of prosthetic implantation is pain or pressure necrosis caused by the tube itself. The dissolution of the tube due to material failure appears to have been largely eliminated in more recent models. Further disadvantages of the 'bridging tube' are dislocation, tumour overgrowth, or penetration. On the other hand, laser coagu-

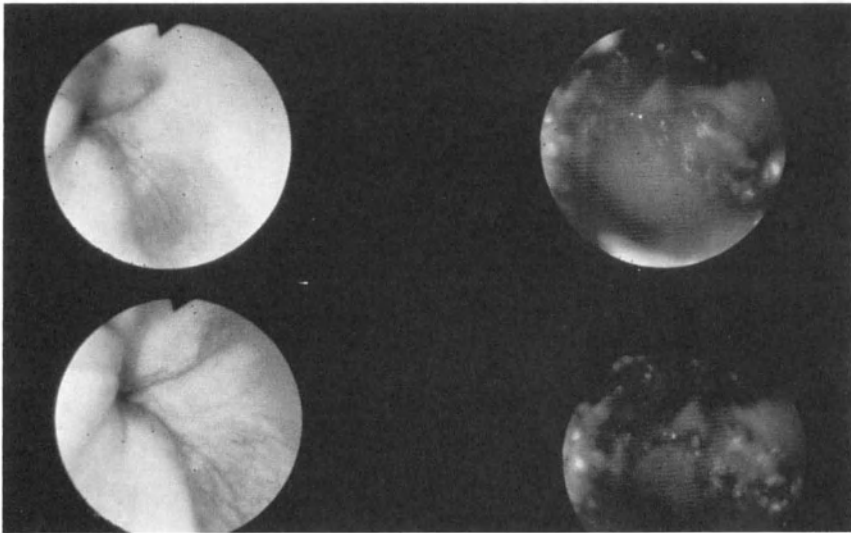


Fig. 4.8 Endoscopic aspect of tumour stenoses of the distal oesophagus before and after laser coagulation

lation offers no possibility for closing an oesophageal fistula. As a rule the opening of the tumour stenosis in the upper gastrointestinal tract by means of the laser beam requires two or three sessions; that is, a period of treatment of 1–2 weeks, while the endoprosthesis can be implanted in a single session. As a general rule laser coagulation needs to be repeated after 3–12 weeks. In addition to the cost of hospitalization, the cost of purchasing and maintaining the laser equipment must also be considered. The principal advantage of the laser is that stenosing tissue is not merely displaced, but rather that patency is re-established by the removal of tissue by vaporization, or the formation of necrosis with subsequent sloughing (Fig. 4.8). Laser coagulation is also possible in the region of the upper sphincter of the oesophagus, although considerable experience on the part of the physician is required (Figs 4.9, 4.10). In complete stenosis that cannot be negotiated even with a thin guidewire, cautious coagulation – working from the proximal margin – can initially be employed to permit the passage of the guidewire.

Before a final evaluation of the two procedures can be made a prospective study is needed, in which mortality and complication

rates, time and cost factors, together with the general well-being (retrosternal pain) of the patient, are the main parameters.

When both methods are available the prosthesis will certainly be given preference in oesophago-bronchial fistulae, in the case of sub-mucous tumour growth, and also possibly in the case of long stenosis (Table 4.8). In contrast, very proximal stenosis of the oesophagus, total or bleeding stenosis with diverging axis of the oesophagus or of very firm consistency, short stenosis with the tumour involving only parts of the circumference or stenosis due to anastomotic recurrences after gastrectomy, would appear to be better dealt with by laser coagulation. This also applies to tumour stenosis associated with concomitant compression of the airways, since the introduction of an oesophageal tube can result in complete occlusion of the trachea or main bronchi.

Depending upon the experience of the endoscopist and on the facilities available, emphasis given to laser or tubes may vary. In principle it is possible to re-establish patency in a high percentage of oesophageal stenosis, through the use of bougienage, chemotherapy or radiotherapy, laser coagulation and the implantation of an endoprosthesis. Almost any of the therapeutic procedures mentioned can be used in combination with others. In general, dilatation is employed to 'prepare the way' for tube implantation or the use of the laser. In squamous cell carcinomas, radiotherapy and chemotherapy can be combined with any other procedure, so that a total of 303 combinations would appear possible. Finally, although the implantation of an endoprosthesis represents an alternative means for treatment of tumour stenosis in the upper digestive tract, the question 'tube or laser?' is already being answered by some authors thus: 'tube and laser'. The future will show which form of treatment or combination of treatments will be given preference for the individual indication.

Colorectal tumour stenoses

In comparison with stenoses of the upper gastrointestinal tract, less experience has been gained with the laser in the colorectum. However, since such non-surgical alternatives as bridging tubes or balloon dilatation are not available, or are still being developed, there is growing

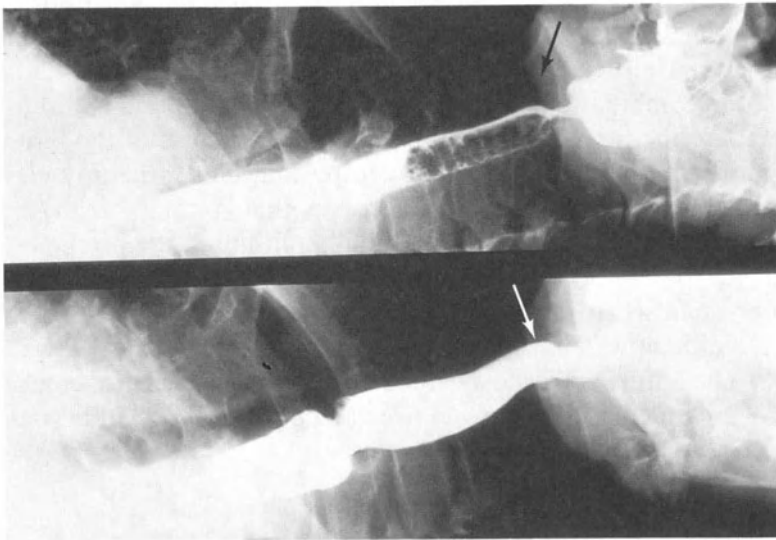
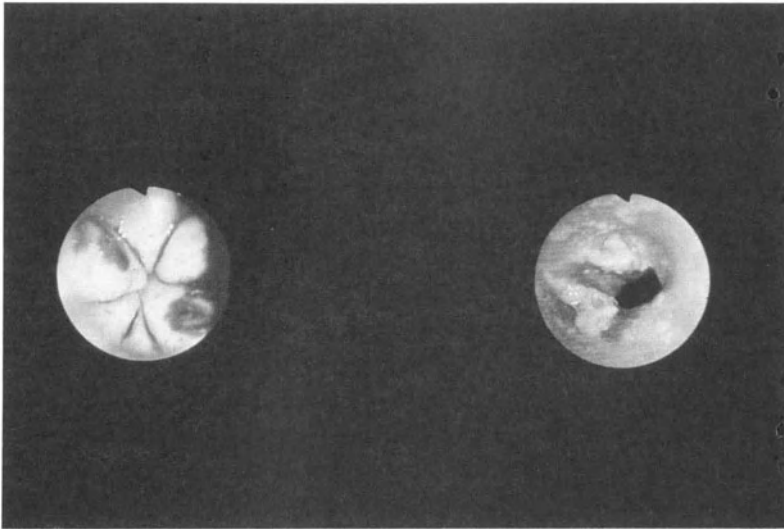


Fig. 4.9 and 10 Endoscopic and radiological aspect of a proximal tumour stenosis (↑) before and after laser therapy

Table 4.8 Tumour stenosis, upper gastrointestinal tract, therapy to be preferred

<i>'Tubes'</i>	<i>Laser</i>
Fistulae	Short stenosis 'Total' stenosis
Tumour length > 6 cm	Rigid stenosis, diverging axis of the stenosis
Submucosal growth	Proximal stenosis—tumour not circumferential Polypoid tumour Postoperative stenosis (oesophago-jejunosomy with recurrence) Tumour compression of respiratory tract

Table 4.9 Follow-up after palliative laser treatment of colorectal tumours⁶²

<i>Period (weeks)</i>	<i>Feature of treatment</i>		
	<i>Obstruction</i> (<i>n</i> = 24)	<i>Bleeding</i> (<i>n</i> = 40)	<i>Obstruction + bleeding</i> (<i>n</i> = 20)
Deceased	8.5 (1–37)	30 (4–150)	19.5 (1–150)
Alive	52.5 (29–67)	52 (19–130)	21.5 (5–34)

interest in colorectal laser application. In contrast to the unchanging incidence of oesophageal carcinoma, colorectal carcinoma is still on the increase.

The indication for laser treatment of colorectal tumour stenosis is restricted to (1) patients who are inoperable due to widespread tumour disease (local or general metastases), or to severe secondary illness; (2) patients refusing surgical treatment; and (3) those presenting with obstruction of sudden onset in the case of left colon cancer. In our experience, laser coagulation is rarely used for haemostasis. Lambert and Sabhen⁶¹ treated 131 patients with colorectal cancer, to the clinical benefit of most of them. Complications were rarely seen in this study. There is a large and well-documented report on laser therapy of colorectal stenoses from Amsterdam⁶². Luminal patency was report-

edly restored in 20/24, and haemostasis achieved in 37/40 patients with colorectal tumour, while 19/20 patients were successfully treated for both bleeding and obstruction. Major complications were seen in 14% of the patients, and comprised perforation (six patients), stricture necessitating colostomy (three patients), and delayed post-treatment haemorrhage (two patients). The laser-related mortality rate was 3.6% (three patients). As in palliative upper GI tract tumour therapy, a wide range of treatment sessions (one to five) and applied energy (980–37 105 joules) proved necessary for effective palliation. The follow-up period varied in accordance with indication, with a longer period for those still under surveillance (Table 4.9).

Among 460 patients with colonic cancer, Kiefhaber⁶³ reported 57 with acute obstruction of the left colon. To avoid a two- or three-stage operation, recanalization of the stenosis was attempted to permit preoperative peroral bowel lavage and primary resection. The stenosis was successfully reopened in 54/57 patients, and two perforations were seen; there was no laser-induced mortality. Twenty-seven patients were operated on, with a mortality rate of 3.7%. Technically, laser coagulation of colonic tumours resembles that of oesophageal tumours. Coagulation is begun on the luminal side and is continued towards the site of origin of the tumour. Some authors prefer to employ pulses of 0.5–1.0 s, and a power setting of 70–100 W. CO₂ flow appears necessary to keep the fibre clean, to temporarily stop bleeding by pressure, and to avoid explosive gas mixtures. However, care must be taken to avoid over-distension of the bowel. In our experience the contact mode of laser application, and the use of a laser-resistant guide, is particularly useful in the colorectum. Results of palliative (and preoperative) laser coagulation appear encouraging – despite the fact that none of the studies reported employed an established therapy protocol, and none incorporated a control group.

Curative tumour therapy

Curative therapy of upper gastrointestinal tumours has been carried out on a large scale in the case of early carcinoma of the stomach, and also, but more rarely, in carcinoma of the oesophagus. In the colon, owing to the fact that growths are usually polypoid, polypectomy is normally the method of choice and curative laser therapy is used

only exceptionally in case of villous adenomas. The use of the laser represents curative tumour therapy in case all cancer cells have been completely destroyed. This is possible only when the following conditions are met:

1. tumour invasion must not involve the full thickness of the gastrointestinal wall; and
2. regional metastases or distant metastases must be excluded.

Since, by definition, these tumours are locally operable lesions, both conditions are usually demonstrated intraoperatively by the resection of the tumour-bearing segment of the bowel and removal of the regional lymph nodes with subsequent detailed histological work-up. The pre-operative diagnostic work-up is not normally capable of showing whether the two preconditions are met. Every locally operable gastrointestinal carcinoma must be treated surgically in accordance with the requirements of cancer surgery. The sole exception is colonic carcinoma within an adenoma, when (1) the carcinoma has been removed with a margin of healthy tissue, (2) it is well or moderately well differentiated, and (3) when no spread to lymphatic or blood vessels can be demonstrated. Potentially curative laser therapy can therefore apply only to patients with locally operable carcinomas who are considered inoperable for general reasons. The establishment of general inoperability, and thus the decision to employ local laser therapy, must be documented jointly by the gastroenterologist and the surgeon.

An important diagnostic aid for establishing the depth of penetration of the tumour, is endoscopic ultrasonography⁶⁴. The endoscopic ultrasonic picture of the wall of the stomach is characterized by five layers. Both real and virtual (border echo) layers are visualized. With the aid of comparative histological examinations, however, the echo-endoscopic layers can be correlated with the histological layers. With a certain degree of reliability the diagnosis of an early gastric carcinoma or early carcinoma of the oesophagus can therefore be confirmed by the echo-endoscopic appearance. The reservation is that the diagnosis is macroscopic and not histological. In no case can lymph node metastases of a carcinoma of the stomach be excluded on the basis of endoscopic ultrasonography. Only in the case of advanced carcinoma of the oesophagus can echo-endoscopy document regional

lymph node metastases with a relatively high degree of reliability.

Early gastric carcinoma The experience of potentially curative laser treatment of gastric carcinoma is restricted to a comparatively small number of patients. The largest group of cases – from Japanese collected statistics – was reported by Takemoto⁶⁵ in 1986. Out of 785 patients with early carcinoma of the stomach, 427 were submitted to endoscopy 1 year after potentially curative laser therapy. A recurrent carcinoma was found in 80 cases (Table 4.10). A breakdown of the 47 early gastric carcinomas submitted to potentially curative laser coagulation by Takemoto *et al.*⁶⁵ revealed five recurrences among the 47 primary lesions 1–5 years after the operation (Table 4.11). Of interest is the breakdown of recurrences by size, macroscopic and histological type, and also by depth of penetration of the initial primary early gastric carcinoma (Table 4.12). Favourable conditions are, apparently, early carcinomas smaller than 2 cm and of type I, when the lesion is a well-differentiated carcinoma limited to the mucosa. In 14 patients, Takemoto *et al.*⁶⁵ carried out echo-endoscopic examinations prior to and after the laser treatment. Although, in all the cases, laser coagulation was considered to be adequate on the basis of the echo-endoscopic image, recurrent carcinoma developed in two of the 14 cases (after 22 and 10 months).

Recently, photosensitizing substances have been employed in the treatment of gastric carcinoma. Kato⁴⁵ reported on the use of an argon dye laser applied after the administration of haematoporphyrin derivatives (HpD). Here the ablating property of the laser is not employed. Rather the cell-destroying effect of photochemical reactions is utilized. Kato⁴⁵ treated 28 patients with carcinoma of the stomach including 17 cases of early carcinoma (10 type II, three IIa, three a combination of IIc and III, and two type I). Among 17 patients (18 lesions), complete remission was achieved in 10 (11 lesions). Among 13 resected carcinomas, complete remission was confirmed histologically in six. In 11 patients with advanced carcinoma, complete remission was achieved in none. Complete remission was defined as the failure to demonstrate residual tumour tissue, either endoscopically, grossly, or histologically; significant remission was defined as the destruction of 60% or more of the original tumour; partial remission

Table 4.10 Potential curative laser treatment of early gastric carcinoma (according to Ref. 65)

<i>Laser</i>	<i>Criteria</i>		
	<i>a</i>	<i>b</i>	<i>c</i>
Nd:YAG	308 (22)	73 (73)	299 (80)
Argon	9 (2)	2 (0)	— —
Ar + HpD	1 (1)	0 (0)	4 (1)
Ar-dye + HpD	28 (4)	2 (0)	50 (14)
Others	1 (0)	3 (0)	5 (3)
	347 (29)	80 (9)	358 (98)

() Number of patients operated on after laser treatment

a = negative biopsy for > 1 year

b = positive biopsy after 1 year

c = pending; laser therapy < 1 year

Table 4.11 Follow-up after potential curative laser therapy of early gastric carcinoma (according to Ref. 65)

<i>n</i>	<i>Follow-up (years)</i>	<i>Cancer in biopsy</i>	
		<i>Positive</i>	<i>Negative</i>
1	5	—	1
11	4	1	10
6	3	—	6
14	2	3	11
15	1	1	14
47			

Table 4.12 Rate of cancer recurrences after potential curative laser therapy (according to Ref. 65)

		<i>n</i>	Cancer	
			<i>Negative</i> (<i>continuously</i>)	Positive
Size	< 2 cm	39	38 (98%)	1
	> 2 cm	8	4 (50%)	4
Macroscopic type	Elevated	→15	15 (100%)	0
	Depressed	→32	27 (84%)	5
Histological type	Differentiated	→43	41 (95%)	2
	Undifferentiated	→ 4	1 (25%)	3
Depth of invasion	Mucosa	31	31 (100%)	0
	Submucosa	16	11 (69%)	5

20–60% of the original tumour; and no remission less than 20% of the primary tumour mass. According to the authors, problems continue to exist owing to the photosensitivity of the skin. Furthermore, difficulties in achieving a uniform distribution of the HpD, or in penetrating into deeper tissue layers, are still encountered. To date it would appear that successful treatment is possible only with small superficial tumours.

In the case of early carcinoma of the stomach, resection done in accordance with the requirements of cancer surgery continues to offer the best chance of achieving a cure. As experience gained to date shows, only in patients considered inoperable for general reasons is laser coagulation of the early gastric carcinoma in curative intent permitted; an echo-endoscopic surveillance is desirable. In any case, regular endoscopic and bioptic follow-up examinations must be ensured.

Benign strictures – benign tumours (precancerous lesions)

So far there are only a few anecdotal reports on the use of laser therapy to treat benign strictures^{60,66}. The best results appear to be obtained in the case of post-operative stricture. Little experience has been gained to date with the treatment of stenoses in Crohn's disease. Controlled

studies comparing laser and conventional dilatation therapy are not available.

Potentially curative laser treatment of precancerous lesions has been reported in the case of gastric and colorectal adenomas⁶⁷⁻⁷⁰.

Rösch and Frühmorgen⁶⁷ reported argon laser treatment in 3/12 patients with flat adenoma of the stomach ('borderline lesion') following snare-ectomy of the premalignant lesion. Kato⁶⁹ employed Nd:YAG laser coagulation to treat 14 broad-based 'borderline lesions' in 12 patients; no complications were observed. Post-laser ulceration healed within 4-7 weeks and afterwards no atypical epithelium was to be seen.

A decision to employ laser therapy in the treatment of precancerous lesions has, however, greater significance in the colorectum. There are also more anecdotal reports on laser ablation of colorectal polyps. The largest and best-documented study, covering 100 patients, was published by Mathus-Vliegen and Tytgat⁷⁰.

Among 26 patients with extensive adenoma growth (more than two-thirds of the circumference, or longitudinal extension more than 4 cm) complete tumour ablation was achieved in only eight, as determined by endoscopic and histological criteria. Relief of symptoms (diarrhoea, excessive mucus discharge, haematochezia, iron deficiency anaemia, faecal incontinence, hypokalaemia and dehydration) was accomplished in 15/16 patients. Follow-up over a median of 94 weeks revealed recurrence/persistence of adenomatous tissue in 18 patients; in five patients carcinoma was detected in biopsy material obtained from the ground of the laser ulcer. Major complications occurred in 11.6% of this group, stenosis in 7.7% and perforation in 3.9%.

Among 22 patients with adenomas of between 1 and 4 cm in diameter, a high percentage of symptomatic relief was achieved (13/22 patients). After a median follow-up period of 44 weeks, complete ablation was observed in 10/22 patients. Carcinoma was demonstrated in five patients; post-treatment haemorrhage occurred as a complication in 9%.

The results obtained in patients with adenomas less than 1 cm were better. Adenoma recurrence/persistence was found in 1/19 patients after an overall follow-up period of 16 weeks; no malignancy was discovered, and no major complication occurred.

In conclusion, it may be said that in adenomas more than 1 cm the

symptoms were very effectively controlled, but sustained ablation of neoplastic tissue was accomplished in only a moderate 40% of the cases. Major complications occurred in about 10% of the patients; carcinoma was detected after laser therapy in 10/40 (25%) of the larger adenomas.

In a second group comprising 21 patients with familial polyposis submitted to subtotal colectomy and ileorectal anastomosis, Mathus-Vliegen and Tytgat detected no malignancy after repeated laser therapy within the rectal stump (follow-up 5 years).

In the case of precancerous lesions, laser coagulation is performed at lower energy levels, that is, 40–50 W). There has been no definitive decision on whether to use a stippling (dotted) or a sweeping, 'paint-brush' technique for laser application; the latter technique does, however, appear to induce more scarring and stenoses.

On the basis of the results presented by Mathus-Vliegen and Tytgat, it must be concluded that laser therapy of benign adenomas, in particular those with a diameter over 1 cm, should be restricted to patients who are inoperable for general reasons. This will apply until certain questions have been answered; for example, how deeply the lesions must be destroyed, or how malignancy in large villous adenomas is to be detected.

A further prerequisite for laser therapy in the lesions mentioned is that close follow-up must be guaranteed both by the endoscopist and the patient.

The major drawback of laser therapy is the lack of a complete histological examination. The result is that the endoscopist is (often) not aware whether he is dealing with a benign precancerous lesion, or with a frank carcinoma. Whenever possible the aim must be a total 'biopsy' and histological work-up, whether by trans-abdominal or trans-anal surgery (method according to Buess). A further drawback of laser coagulation is that subsequent excision of the mucosal lesion is impaired by scarring. In the case of inoperable patients, laser therapy of benign precancerous lesions has produced promising results, but the persistence/recurrence rate seems to be higher (40%) than after surgery (17%)⁷¹. According to Tytgat⁷² there is still no evidence that laser therapy of benign precancerous lesions represents progress in the case of the patient with an average surgical risk.

COAGULATIVE LASER THERAPY

Gastrointestinal bleeding

The initial application of the laser in the gastrointestinal tract was the treatment of active or potential bleeding. Goodale⁸⁰ was the first author to report on successful haemostasis with the CO₂ laser in animal experiments.

The development of laser coagulation as a technique for stopping bleeding underwent several phases. The first phase was characterized by reports on experiments carried out on animals. Here, for the most part, the argon and Nd:YAG laser were employed^{73-81,83}. The second phase was characterized by case reports of laser coagulations in the gastrointestinal tract^{2,82,83a}. The third phase followed with reports on larger numbers of patients in whom haemostasis by laser coagulation had proved successful^{25,26,84,85}. The compilations were impressive because of the large number of patients involved, and the high rate of success achieved. In most cases no information was provided on re-bleeding rates. Since these studies were 'uncontrolled', conclusions as to the effect of laser coagulation on such prognostic factors as mortality, length of hospitalization, incidence of operation or transfusion rates cannot be drawn with any degree of certainty. Despite these reservations, the commercialization of laser technology for use in gastrointestinal haemostasis began in this phase. Therefore controlled studies, which were initiated in the fourth and last phase, have been carried out only to a limited extent. So far, comparative investigations have been reported only in references 86-96. With the exception of Fleischer³¹, who coagulated patients with acute oesophageal variceal bleeding, most of the other studies are concerned primarily with haemorrhages from peptic lesions.

The discussion as to which laser would be best suited for haemostasis in the gastrointestinal tract arose at an early date. Since no flexible transmission system was available for the CO₂ laser, attention was concentrated on the argon and the Nd:YAG lasers. A major argument in favour of the argon laser was its maximum absorption in the red-green range. As a result of this property, the depth of penetration of this laser is smaller than that of the Nd:YAG laser. Animal experiments suggested that the argon laser would be associated with a lower complication rate and greater safety^{73,74}. The higher energy level of

the Nd:YAG laser promised more effective haemostasis^{37,81}. It is very likely that future discussions of this topic will include other lasers with additional properties with respect to the most commonly employed laser systems to date – the argon and Nd:YAG lasers – the following remarks can be made: both lasers can control gastrointestinal bleeding; the argon laser is possibly somewhat safer, although in controlled studies a remarkably low complication rate has also been reported for the Nd:YAG laser. As a result of the greater energy applied, the use of the Nd:YAG laser appears somewhat easier, since haemostatic effects can still be achieved from a greater distance than with the argon laser. To date, the majority of laser users have decided in favour of the Nd:YAG laser system.

Method Before attempting to accomplish endoscopic haemostasis the location and differential diagnosis of a bleeding source must be established within the framework of an emergency endoscopy. A further task of emergency endoscopy is to differentiate between an actively bleeding lesion (Forest Ia and Ib), and bleeding sources with stigmata of recent bleeding. The latter are lesions with stigmata of prior bleeding (Forrest II). Of particular importance here is the visible blood vessel, which will be discussed later. Nowadays, oesophageal varices are first treated by sclerotherapy. On account of frequent recurrent bleeding in the short term, laser coagulation has not been accepted for use in this situation.

Among almost 1500 reviewed emergency endoscopies done at the Medical Department of the University Hospital at Erlangen, combined lesions were observed in about 20% of the emergency endoscopies performed. More than two-thirds of the cases with verified sources of bleeding had peptic lesions. The distribution of potential bleeding sources roughly corresponds to that of actively bleeding sources (Table 4.13).

More than 90% of the authors employ the high-energy Nd:YAG laser. As described above, generally either a conventional fibreglass instrument (e.g. GIF Q10, Olympus) is used, or a treatment modification with a larger biopsy channel. During haemostasis a constant flow of CO₂ is recommended to cool the quartz fibre and to remove liquid blood.

Successful haemostasis by laser coagulation results in closure of the

Table 4.13 Emergency Endoscopy, Medical Department, University Hospital, Erlangen

Verified sources of bleeding (Forrest I, II) (<i>n</i> = 1424)		62.5%
Solitary	48.4%	
Multiple	14.1%	
Potential sources of bleeding (Forrest III)		26.5%
Solitary	19.0%	
Multiple	7.5%	
Undetected source of bleeding in unequivocal upper GI haemorrhage		4.0%
Normal findings		7.0%
Verified source of bleeding (Forrest I, II) (<i>n</i> = 878)		
Gastric ulcer, anastomotic ulcer		23.3%
Duodenal ulcer, duodenal erosions		22.0%
Esophageal/fundic varices		15.1%
Esophagitis		13.4%
Gastric erosions		12.8%
Mallory-Weiss syndrome and other rarer lesions		8.3%
Gastric tumours		5.1%

bleeding vessel. Application of the laser energy directly to the flowing blood results in the rapid transport of the energy – now converted into heat – away from the site of application, so that insufficient energy is available for occluding the vessel. Therefore a circular area around the bleeding vessel should first be coagulated. This way the supply of blood via the afferent limb of the vessel can be reduced, or even prevented. The subsequent coagulation of the vessel itself is usually achieved with little effort.

In our experience, the injection of adrenalin 1 : 10 000 or 1 : 100 000, as first suggested by Soehendra⁹⁸, has proved useful. With this method, Forrest Ia or Ib haemorrhages can often be arrested, which improves the preconditions for final laser coagulation.

In particular, in the presence of visible vessels, coagulation is often accompanied by an intensification of the bleeding. Not infrequently a Forrest II lesion is thereby converted into a spurting (Forrest Ia) haemorrhage. Here, too, prior injection therapy proved useful in our experience. However, to date no reports on comparative studies – including animal experiments – of laser coagulation alone, and laser coagulation after previous injection, have been published.

Results A number of uncontrolled studies^{84, 85}, have demonstrated that successful haemostasis can be accomplished with laser coagulation. A poll conducted by Kiefhaber revealed that, among 1729 patients, haemostasis was successfully accomplished in an average of 84% (70–100%) of the cases. No data on re-bleeding were presented. In 1982, in a total of 994 cases of acute bleeding that occurred in 625 patients from a variety of bleeding sources (ulcers, erosions, vascular anomalies, bleeding tumours, Mallory-Weiss tears, and varices), Kiefhaber²⁵ reported successful haemostasis in 94% of cases. Patients with chronic ulcers seemed to profit by a reduction in the lethality rate, while in the case of drug- and stress-induced ulcers the situation was less favourable. The decisive prognostic factor for the overall mortality rate was the age of the patient^{98a}.

One of the first controlled studies was published by Rutgeerts in 1982⁹⁴. In an initial – admittedly uncontrolled – part of this study involving Forrest Ia bleedings (23 patients), a haemostasis rate of 87% was achieved which, however, was associated with re-bleeding in 55% of the cases. The operation rate, at 61%, was appreciably lower than that of a control group from an earlier study, namely 95%. However the mortality rate in the laser-treated group was 30%. In the second, controlled part of Rutgeerts' study (86 patients with active non-spurting bleeding), laser coagulation showed significant influence on haemostasis (100% versus 77%), on re-bleeding rate (7% versus 20%), and on the incidence rate of surgery (2% as compared with 13%). In the coagulated group the mortality rate was 13%, in the control group 15%; the difference was not statistically significant (Table 4.14). In a study by MacLeod in 1982⁹³, the use of the Nd:YAG laser significantly reduced the re-bleeding rate and the incidence of emergency operations. In contrast, however, the transfusion rates, the duration of hospitalization, and mortality rates were not significantly influenced by laser coagulation.

The study carried out by Swain in 1983⁹⁵ revealed a significant effect of laser coagulation on the haemostasis rate, and also on the mortality rate of the laser-treated patients, which was significantly reduced. In the studies by Ihre⁸⁸, Escourrou⁸⁶ and Krejjs⁹⁶, no significant effect of laser coagulation was determined.

In addition to the already mentioned investigations with the Nd:YAG laser, three more controlled studies with the argon laser

Table 4.14 Laser coagulation in active nonspurting gastroduodenal bleeding (according to Ref. 97)

	<i>Laser</i>	<i>Control</i>	
No. of patients	46	40	
Bleeding stopped	46 (100%)	31 (77%)	<0.001
Bleeding recurred	3/46 (7%)	6/31 (20%)	<0.1
Operation	1 (2%)	5 (13%)	<0.1
Overall mortality	6 (13%)	6 (15%)	NS

have been published. In the trials carried out by Swain⁸⁹, out of 330 patients submitted to emergency endoscopy, 76 patients with endoscopically observable gastroduodenal ulcers and stigmata of recent bleeding were included in the study. Fifty-three ulcers had a visible vessel. Eight out of 24 laser-coagulated, and 17 out of 28 control patients experienced re-bleeding. In the control group, seven patients died; none in the laser-coagulated group – a difference which proved statistically significant. In a study involving 136 patients, Vallon⁹⁰ was unable to find any significant reduction in recurrent bleeding, incidence of surgery or mortality rate. In a small trial involving 12 patients, Jensen⁹² observed initial haemostasis in 100%; through laser coagulation, the rate of emergency operations and the rate of transfusions were significantly reduced.

Before evaluating the results of controlled studies of photo-coagulation of gastrointestinal bleeding, the prognostically important factors will be considered. Bordley⁹⁹ has once again summarized the clinical conditions carrying an unfavourable prognosis. These included an age of more than 75, severe secondary diseases, ascites, a drop in blood coagulation, systemic blood pressure below 100 mmHg (1 h after admission to hospital) and continuing haemorrhage (red blood in the gastric tube). The fact that continuing bleeding on admission to the hospital is associated with a four to twelve times higher mortality rate than bleeding that has stopped, was already reported by Jones in 1956¹⁰⁰, Schiller in 1970¹⁰¹, and Jones in 1976¹⁰². The large number of variable parameters involved has so far made it impossible for the effect of emergency endoscopy on the prognosis of patients with gastrointestinal haemorrhage to be unequivocally demonstrated^{103, 104}.

The large majority of patients with gastrointestinal bleeding can expect no improvement in the prognosis from emergency endoscopy, since only about 15–25% of the patients are actively bleeding during this examination. Only 18%¹⁰⁵ to 48%¹⁰⁶ reveal a so-called visible vessel and according to these authors, have a re-bleeding rate of 56–85%. Wara¹⁰⁷ reported a re-bleeding rate in patients with a visible vessel of less than one-third; however, in his report further endoscopic criteria can be defined as risk factors. Additional oozing, coagula overlying the visible vessel, or location of the ulcer with 'visible' vessel in the stomach or duodenum (in contrast to a pre-pyloric ulcer). Although a number of further stigmata can be taken as signs of an increased potential recurrent bleeding, in controlled studies on laser coagulation, stratification in accordance with Forrest¹⁰⁸ has proved useful.

To summarize the results of the controlled trials, it is striking that perforations have apparently not occurred (in seven out of nine studies), while in the uncontrolled trials the perforation rate is 1–2%. Primary haemostasis was higher in uncontrolled studies (up to 90–100%). In some investigations laser coagulation appears to have a positive effect on haemostasis, the re-bleeding rate, transfusion rate and mortality rate. However, the improvement in the prognosis by laser coagulation of gastrointestinal haemorrhage is by no means in the range that the results of the uncontrolled studies lead us to expect. In any case, further controlled trials are necessary. It must, however, be stressed that in the (controlled) studies laser coagulation has always been compared with conservative treatment. Of importance for clinical practice, however, would be a comparison of laser coagulation with (emergency) surgery.

For clinical practice the following procedure is recommended: in the case of Forrest Ia bleeding, as a rule, immediate surgery should be the aim. Those with adequate experience with laser coagulation can attempt haemostasis, with the aim of postponing surgery to the bleeding-free interval rather than performing emergency surgery. In the case of continuing Forrest Ib bleeding, also, endoscopic haemostasis can be attempted, with its associated chances of achieving a significant improvement with respect to re-bleeding and the incidence of emergency surgery. However, a reduction in mortality has not been proved. As a rule, Forrest IIa bleeding without visible vessel should be treated conservatively: in the presence of a visible vessel, laser

coagulation is associated with a significant improvement in mortality. Here, too, however, the alternative to laser therapy is surgery. Large ulcers not completely to be seen endoscopically, and ulcers located at the posterior wall of the duodenal bulb, represent the worst condition for endoscopic haemostasis. In the case of bleeding from an ulcer, a re-bleeding rate within the first 2–3 years of about 60% must be expected. For this reason, in operable patients haemostasis must always be accompanied by treatment of the ulcer disease.

Admittedly, a significant number of patients cannot be incorporated into controlled trials for ethical reasons. These are patients who cannot be referred to surgery on account of severe secondary diseases. Here, laser coagulation is promising in the treatment of acute gastrointestinal bleeding and, of course, is also justified as the only method available, although the results of controlled studies will give rise to controversial discussion.

Alternative haemostatic procedures Attempts to arrest endoscopically diagnosed bleeding with the aid of haemoclips or fast-setting resins proved to be ineffective. Alternative procedures to photocoagulation are electrocoagulation and the injection of various substances.

Electrocoagulation has the advantage that the equipment employed is transportable, i.e. mobile, and the costs compared to laser coagulation are considerably lower. In 1981 Papp¹⁰⁹ reported an 88% success rate for haemostasis with monopolar electro-coagulation. The study was, however, uncontrolled, and such sources of bleeding as Mallory–Weiss tears and gastritis had been included. In another uncontrolled trial on the use of bipolar electrocoagulation, Donahoe¹¹⁰ achieved initial haemostasis in 11 out of 14 patients, two of whom, however, experienced recurrent bleeding within 48 h. In 1984, Kernohan¹¹¹, also using bipolar electrocoagulation, was unable to report any reduction in recurrent bleeding, transfusion rate or incidence of surgery. According to Matek¹¹², in uncontrolled trials, haemostasis achieved with electro-hydrothermal coagulation was comparable to that accomplished with laser coagulation.

With the injection of adrenalin at concentrations of 1:10 000 or, in our own experience, 1:100 000, or of polidocanol⁹⁸ haemostasis, comparable to that produced with laser coagulation, can be achieved. The alternative procedures listed here have the advantage of lower

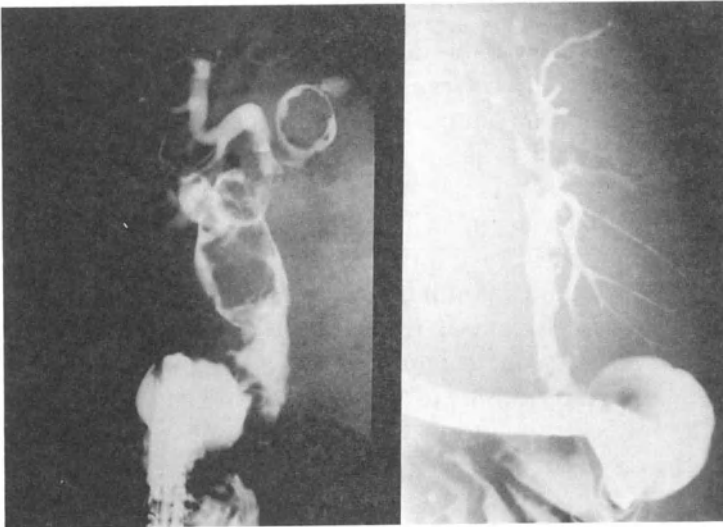


Fig. 4.11 Giant common bile duct concrement after choledochoduodenostomy before and after laser lithotripsy under visual control

cost and greater mobility. Although, on the basis of clinical experience, they appear to be equally as successful as the laser technique, their effectiveness has yet to be proven in controlled trials. The same applies to the heater probe developed in recent years¹¹³.

GALL STONE LITHOTRIPSY

In 1983 Orii *et al.*¹¹⁴ reported the first successful destruction of common bile duct stones with the aid of the Nd:YAG continuous-wave laser in man using transcutaneous-transhepatic cholangioscopy. Later Kouzu¹¹⁵ pointed out that contact laser lithotripsy with sapphire tips was particularly suitable for the destruction of cholesterol stones. Mills and co-workers¹¹⁶ reported *in vitro* experiments of gall stone lithotripsy. As far as our own experiments are regarded the continuous-wave Nd:YAG laser seems ineffective for lithotripsy of gall stones. Only thermal melting and 'drilling' effects, but no fragmentation, could be observed.

With the aid of a flashlamp-pulsed Nd:YAG laser we succeeded^{117,118}, for the first time, in reliably and reproducibly destroy-

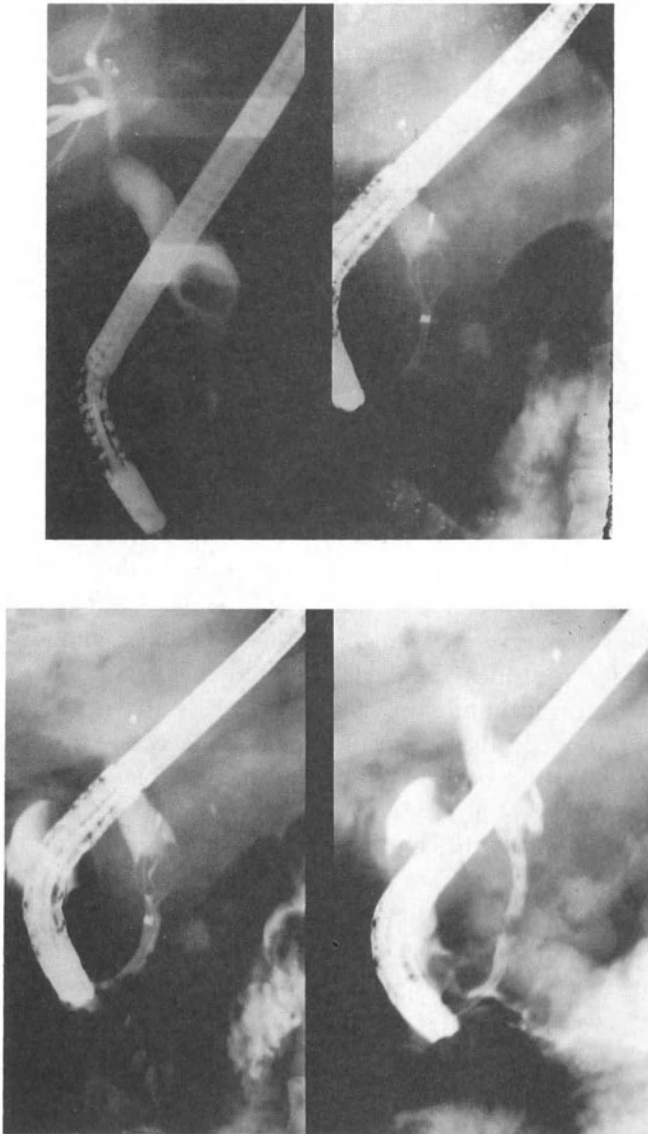


Fig. 4.12a-d Common bile duct concrement, distal narrowing of the common bile duct (12a); Dormia basket grasping the concrement (12b), lithotripsy splitting the concretions in two parts (12c) and concrement-free c.d. (12d)

ing gall stones. After *in vitro* tests animal experiments were performed. The energy required for fragmentation was between 10 and 200 joules, the pulse duration in the millisecond range, and the pulse energy between 0.15 and 2 joules. The time required to fragment the stones varied between 2 and 60 seconds. The lithotriptic effect is of a thermal nature and can be explained by the high vaporization pressures produced within the stone.

The laser light is transmitted via highly flexible glass fibre with a diameter of 0.2 mm without appreciable transmission losses, even when the fibre is strongly flexed. This was one prerequisite for endoscopic retrograde contact laser lithotripsy via conventional duodenoscopes or choledochoscopes (mother-and-baby scope).

Similar thermal lithotripsy effects can be achieved with a flashlamp-pulsed dye laser¹¹⁹. With this system, however, higher energy densities have to be applied.

Initial attempts to accomplish lithotripsy of gall stones with the aid of the Q-switched Nd:YAG laser failed due to the impossibility of coupling high pulse energies with a flexible glass fibre¹²⁰. The use of smaller pulse energies proved to be more effective, but the attempts to break down stones regularly resulted in the destruction of the necessary opto-mechanical coupling system located at the distal tip of the light guide¹²¹. In the meantime we have succeeded in coupling high pulse energies of up to 50 mJ at pulse times in the nanosecond range, with a flexible glass fibre, and have been able to destroy gall stones reliably and reproducibly without any damage occurring to the distal focusing facility.

The advantage of the Q-switched laser system over the flashlamp-pulsed Nd:YAG laser is that thermal injuries to the bile duct need not be feared.

Recently we succeeded for the first time in accomplishing endoscopic retrograde laser lithotripsy of giant bile duct concrement (Figs. 4.11 and 4.12), using a pulsed Nd:YAG laser¹²². To ensure a safe distance of the quartz fibre tip from the wall of the common bile duct, various techniques, involving the use of a Dormia basket, a balloon catheter, or direct visual endoscopic control (Fig. 4.13), have been developed.

At the present time it is not possible to predict which laser system will prove useful in practical application. It is, however, to be expected that laser lithotripsy will become a preferred complementary pro-

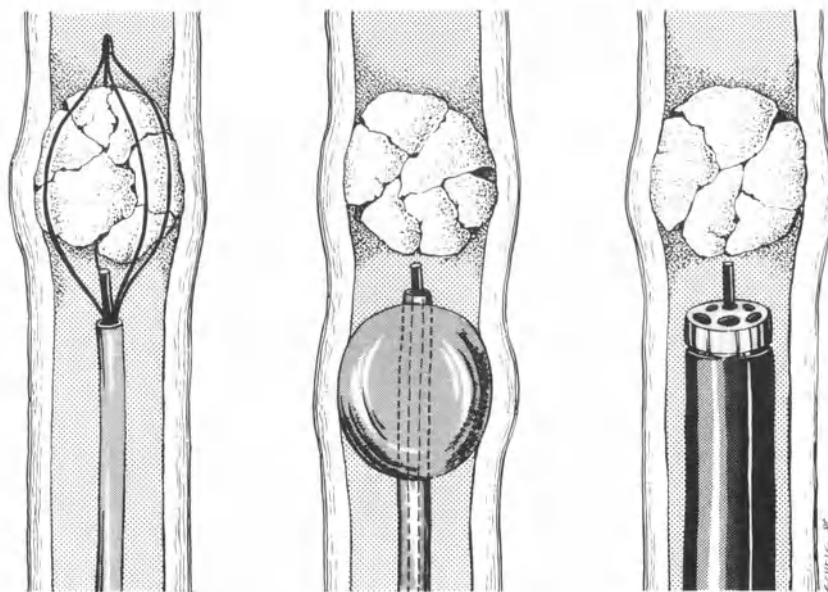


Fig. 4.13 Laser-lithotripsy by aid of (1) a Dormia basket with central laser-fibre (left); (2) a balloon catheter to avoid tissue contact of the laser fibre (middle) and (3) direct endoscopic control (right)

cedure to endoscopic papillotomy in the treatment of large common bile duct stones.

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5

Endoscopic Sonography of the Upper Gastrointestinal Tract: the Present Position

H. Dancygier and M. Classen

The development of morphologically oriented methods has considerably increased our diagnostic capabilities. Sonography plays a leading role in the diagnosis of hepatobiliary, pancreatic and gastrointestinal diseases. It may be used for screening purposes as well as in frequent follow-up investigations. Despite this progress there still remain regions that cannot be accurately depicted even with the most sophisticated imaging modalities presently available. The wall of the gastrointestinal tract and its immediate surroundings may be seen on computed tomographic scanning and with conventional ultrasound, but the systematic and detailed examination of these structures with transcutaneous ultrasonography and computerized tomography (CT) is not possible.

DEVELOPMENT OF ENDOCAVITARY SONOGRAPHY

The desire to close this diagnostic gap led to early attempts to introduce ultrasonic probes working with high frequencies into the body in the hope of obtaining a detailed picture of the gastrointestinal wall and of adjacent tissues. In 1967 the human prostate was demonstrated by the transrectal route using an ultrasonic probe attached on the tip of a rigid rectoscope¹. The development of endoscopic ultrasound

tomography of the upper gastrointestinal tract was initially characterized by attempts to introduce tiny ultrasonic probes through the biopsy channel of a flexible gastroscope and by placing then the tip on the desired position of the gastric mucosa². These probes created an A-scan that allowed differentiation of large cystic lesions from solid alterations. However, since the diagnostic value of the one-dimensional A-scan is very limited this type of endoscopic ultrasonography did not achieve any clinical importance.

For the first time, in 1980, gastroscopes were used with an ultrasonic probe incorporated in the tip, which generated a B-scan^{3,4}. These probes were either composed of linear ultrasonic elements or they had a rotating reflector that created a sector scan. Subsequently the sector scan has achieved major importance in the endosonographic diagnosis of the upper gastrointestinal (GI) tract, although linear working devices in combination with forward-viewing endoscopes may also be used conveniently in selected clinical situations⁵.

In the first prototypes the rigid end of the instrument measured 8 cm, and they worked with a frequency of 5 MHz and generated an 85° scan. The latest generation of instruments (Olympus Opt./Aloca; GFU M2/EUM 2) is characterized by an ultrasonic probe only 2 cm long with a diameter of 1.3 cm (Fig. 5.1). The device works with a frequency of 7.5 MHz and generates a 360° endosonographic round-view (Fig. 5.2) arranged perpendicular to the long axis of the endoscope. Table 5.1 summarizes the main technical features. At the present time we are also evaluating scopes that work with a frequency of 10 MHz.

TECHNIQUE OF INVESTIGATION

The patient is premedicated and examined in the left lateral decubitus position. The scope is introduced under endoscopic control into the descending duodenum. The sonographic examination is performed by slowly pulling back the tip of the instrument, and at the same time rotating the endoscope around its long axis. The sonographic image is continuously followed on a monitor. The prerequisite for a satisfactory image is the undisturbed transmission of the ultrasonic beams. This may be achieved in two ways. On the one hand a rubber balloon may be mounted around the sonographic probe, containing about 10 ml of

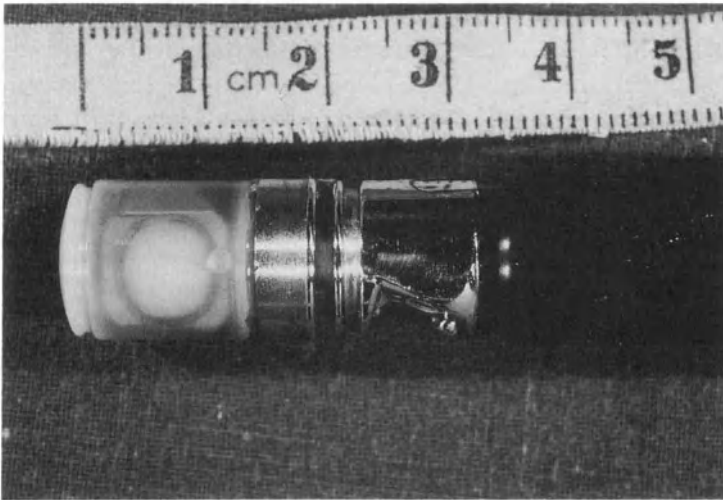


Fig. 5.1 Ultrasonic upper GI endoscope GFU M2/EUM 2 (Olympus Opt./Aloca)

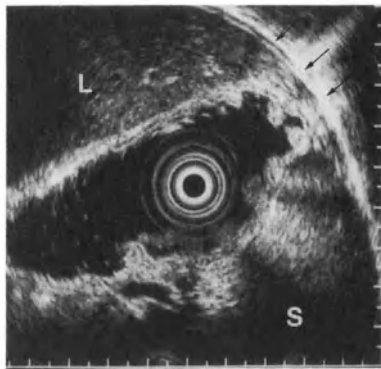


Fig. 5.2 Endosonographic panoramic view from the stomach: L=liver; S=spleen, diaphragm (arrows)

water, thus creating a segment of facilitated ultrasonic beam transmission. This technique is used in the duodenum and prepyloric antrum, as well as in the oesophagus. Investigation of the remaining parts of the stomach is performed after filling this organ with about

Table 5.1 Technical features of the ultrasound endoscope GFU M2/EUM 2 (Olympus/Aloca)

Ultrasound frequency (MHz)	7.5
Rotations (per second)	10
Sector scan (°)	360
Length of rigid end (cm)	4.2
Length of ultrasonic probe (cm)	2
Diameter (cm)	1.3

200 to 300 ml of deaerated water. The probe should be moved to a distance of about 1–2 cm from the gastric wall to keep it in focus. In the stomach this water immersion technique can be combined with the balloon method (Fig. 5.3).

In addition to movements of the endoscope the patient may be placed into different positions to achieve good demonstration of the region of interest. Endosonography of the upper GI tract is a dynamic investigation, and the tip of the instrument is in constant movement due to the peristaltic waves of the stomach and duodenum, the patient's respiratory movements and referred pulsations of the large vessels. Rotation of the tip of the instrument leads to an inversion of the image. These factors make orientation and evaluation of the image difficult. Therefore this technique should be used only by a physician who is well trained in endoscopy and conventional sonography. The duration of the endosonographic investigation should not exceed 30 minutes.

We will first describe the normal findings, and then concentrate on typical endosonographic patterns of pathological lesions in the upper GI tract. Thereafter we will examine the efficacy of this method in selected disorders, and based on these data we will try to elaborate the indications for intraluminal ultrasound of the upper GI tract.

OESOPHAGUS

Due to its tubular form the oesophagus is well suited for endosonographic examination. The evaluation of its wall and the adjacent structures is quite easily accomplished without problems of orientation⁶. The normal oesophageal wall measures about 4–5 mm and displays a typical three-layer pattern. The middle relatively broad

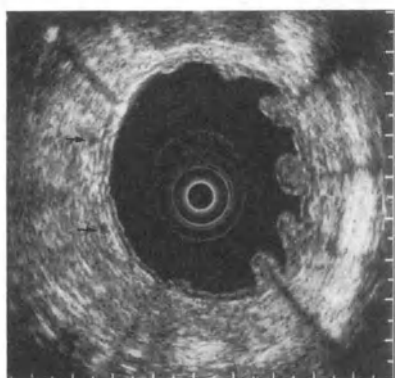


Fig. 5.3 Combined investigation technique in the stomach. Normal gastric wall shown. Note shadows behind gastric folds. Paragastric veins (arrows).

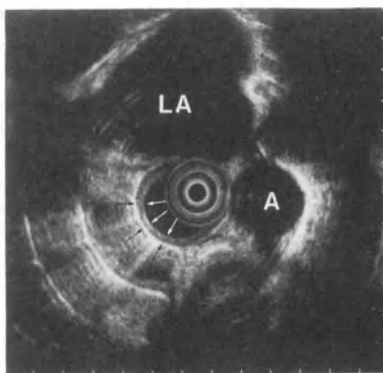


Fig. 5.4 Normal oesophageal wall (between arrows): A = aorta; LA = left atrium

echo-poor layer is believed to correspond to the T. muscularis propria, and is lined by two narrow echogenic bands (Fig. 5.4).

The exact evaluation of the integrity of this echo-poor layer is of great importance for the preoperative staging of oesophageal carcinoma into the prognostically different tumour stages pT2 or pT3. In order to delineate even small alterations of the oesophageal wall the balloon method has to be employed in the examination of the gullet. By doing so compression of intraluminal lesions, e.g. oesophageal varices, is quite frequent, and these dilated vessels may therefore escape endosonographic imaging. However, the demonstration of intramural varices is not affected by inflating the balloon (Fig. 5.5). Oesophageal carcinomas lead to a loss of the normal layer pattern, and to a localized or diffuse thickening of the wall. If tumour stenosis can be passed with the scope the longitudinal extension can be judged, and the transmural view also allows determination of the extension in depth. The penetration of the tumour into the pericardium or into the aorta may be shown or excluded by endosonography (Fig. 5.6). Paraoesophageal echo-poor, round or oval lesions correspond to enlarged lymph nodes and may serve as further evidence of the malignant genesis of the oesophageal lesion. However, in our opinion the sonographic echo pattern does not allow differentiation between

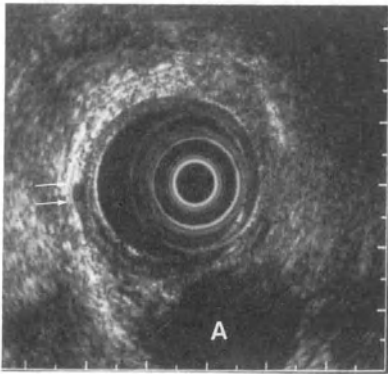


Fig. 5.5 Cross-sectioned oesophageal intramural varicose vein (arrows). On endoscopic examination oesophageal varices °II were seen, that are compressed by the water-filled balloon on endosonographic investigation: A = aorta

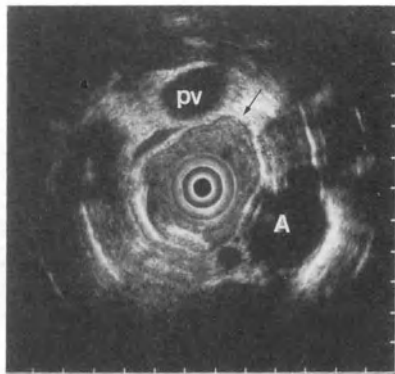


Fig. 5.6 Oesophageal carcinoma. Diffuse thickening of the oesophageal wall with an irregular echo pattern. The outer limits are undulated but sharp (arrow): pv = pulmonary vein; A = aorta

benign hyperplastic and metastatically enlarged lymph nodes. Apart from the evaluation of the oesophageal pathology proper, the trans-oesophageal view offers an excellent sonographic window for the demonstration of the adjacent structures. Part of the mediastinum may be evaluated, and alterations of the left atrium, atrial septum, the mitral valve and of the pulmonary veins may be shown⁷.

STOMACH

The wall of the stomach may be demonstrated in all parts of the organ, but the corpus is especially well suited. The wall thickness is about 4–6 mm. Endosonographically a five-layer pattern may be discriminated. The inner layer is composed of an inner echogenic and an outer echo-poor band. These sonographic layers are caused by impedance changes from the water-filled lumen to the mucosa with its mucous cover. The middle echogenic layer is probably generated by submucosal structures, whereas the muscular coat of the stomach and the serosa present as an echo-poor and a slim outer echogenic band respectively. The latter is not regularly depicted. If the ultrasonic beam hits a gastric

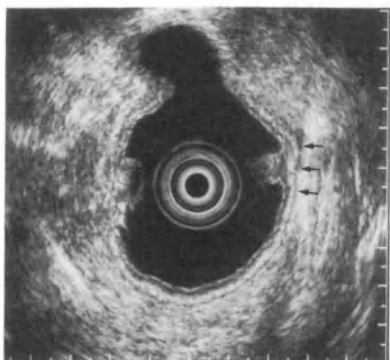


Fig. 5.7 Two polypoid lesions in the stomach. The normal gastric layers are destroyed but the polyp on the right (arrows) does not progress beyond the outer gastric margin, whereas the polyp on the left has destroyed the gastric wall completely and protrudes far into the perigastric tissues and thus fulfils the endosonographic criteria of malignancy.

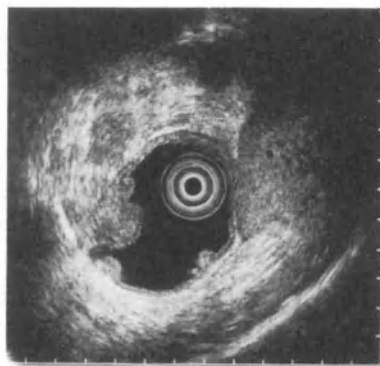


Fig. 5.8 Polypoid gastric carcinoma. Note echo-poor, irregularly lined paragastric areas, which correspond to metastatic lymph nodes

fold perpendicularly it is scattered, and this leads to a shadow behind the fold with a corresponding extinction of dorsal structures (Fig. 5.3).

Knowing this normal sonographic structure of the stomach polypoid tumours, intramural alterations as well as space-occupying lesions pressing from outside on the gastric wall may be discriminated endosonographically.

Benign lesions are sharply demarcated and do not grow beyond the outer gastric layers. The infiltrative growth of malignant processes leads to a destruction of the gastric wall. The typical layering is no longer discernible, the tumour is irregularly lined (Fig. 5.7). In these cases localized paragastric echo-poor areas correspond to metastatic lymph nodes (Fig. 5.8). In the differential diagnosis of these echo-poor lesions dilated gastric veins caused by portal hypertension must be considered. They are usually smaller than lymph nodes and show a typical 'lined-up' appearance (Fig. 5.9). Prominent gastric folds that do not flatten after introduction of air or water, and give the endoscopic

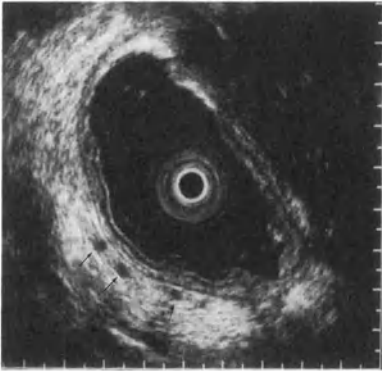


Fig. 5.9 Cross-section of dilated paragastric veins (arrows) in portal hypertension

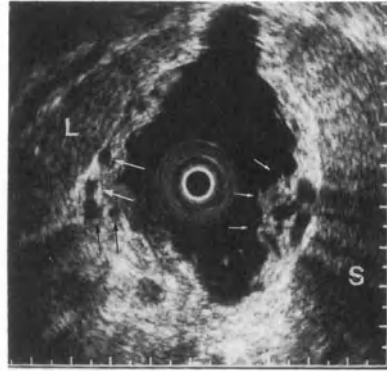


Fig. 5.10 Prominent gastric fold caused by intramural varicose veins (arrows). Between the liver (L) and the stomach wall several cross-sectioned dilated veins are demonstrated (arrows); S = spleen

impression of being caused by malignant infiltration, e.g. by lymphoma, are quite often caused by dilated varicose veins. By permitting a view of the 'core' of such a lesion these findings are rapidly clarified endosonographically, and a potentially deleterious snare biopsy is avoided (Fig. 5.10). Contractions of the gastric wall and oblique scanning may give the false impression of a thickening of the stomach, and may lead the investigator to the wrong diagnosis of a gastric tumour. Such misinterpretations can be avoided by sufficiently distending the stomach with water and by a meticulous interpretation of all sonographic layers.

PANCREAS

The close contact of the pancreas to the stomach and duodenum makes this organ readily accessible to endosonographic examination. The head of the pancreas is best viewed from the duodenum and from the gastric antrum, while the body and tail regions are delineated by slowly pulling back and straightening the instrument in the gastric corpus (Figs. 5.11 and 5.12). The normal pancreas has a regular echo pattern. Even small (<0.5 cm) pseudocysts may be well delineated

(Fig. 5.13). Calcification of the pancreatic parenchyma and intraductal stones are displayed as bright shadowing echoes. The main pancreatic duct may be evaluated in all its segments and even irregularities of its outer margins as well as dilated small branches are shown by endosonography (Fig. 5.14). Nevertheless, it is difficult to differentiate chronic inflammatory from malignant alterations and the delineation of a malignant tumour in a chronically inflamed organ may even become impossible. Pancreatic carcinomas are usually echo-poor and their local growth, as well as metastatic regional peripancreatic and peribiliary lymph nodes, may be evaluated endosonographically (Fig. 5.15)⁸.

GALL BLADDER, BILE DUCTS AND LIVER

The main bile duct may be sharply depicted from the descending duodenum. The dilatation, and often the cause of the obstruction, may be demonstrated. The wall of the common bile duct is composed of three sonographic layers. Stones in the main common bile duct appear as sharp, bright shadowing echoes. The demonstration of space-occupying lesions in the hilum of the liver, as well as of the Klatskin tumour, a carcinoma growing along the bile ducts, are difficult. The gall bladder may be viewed best from the upper portion of the descending duodenum. Even small, tightly packed stones do not produce large irregular echoes, but may be clearly distinguished as individual stones. This sharpness of the picture and the location of the ultrasonic probe close to the gall bladder give rise to the expectation that even tiny polyps of the gall bladder, as well as the infiltrative growth of tumours, will be displayed better, and hopefully earlier, with this method than with conventional techniques⁹.

The parts of the liver near to the ultrasonic transducer are depicted well. This holds especially for the left lobe of the liver and adjacent parts of the right lobe. The predetermined and relatively fixed position of the scope inside the duodenum and the gastric antrum, as well as the low tissue penetration of ultrasonic high-frequency beams, are the main reasons that not all parts of the liver can be evaluated.

SONOGRAPHY OF UPPER GI TRACT

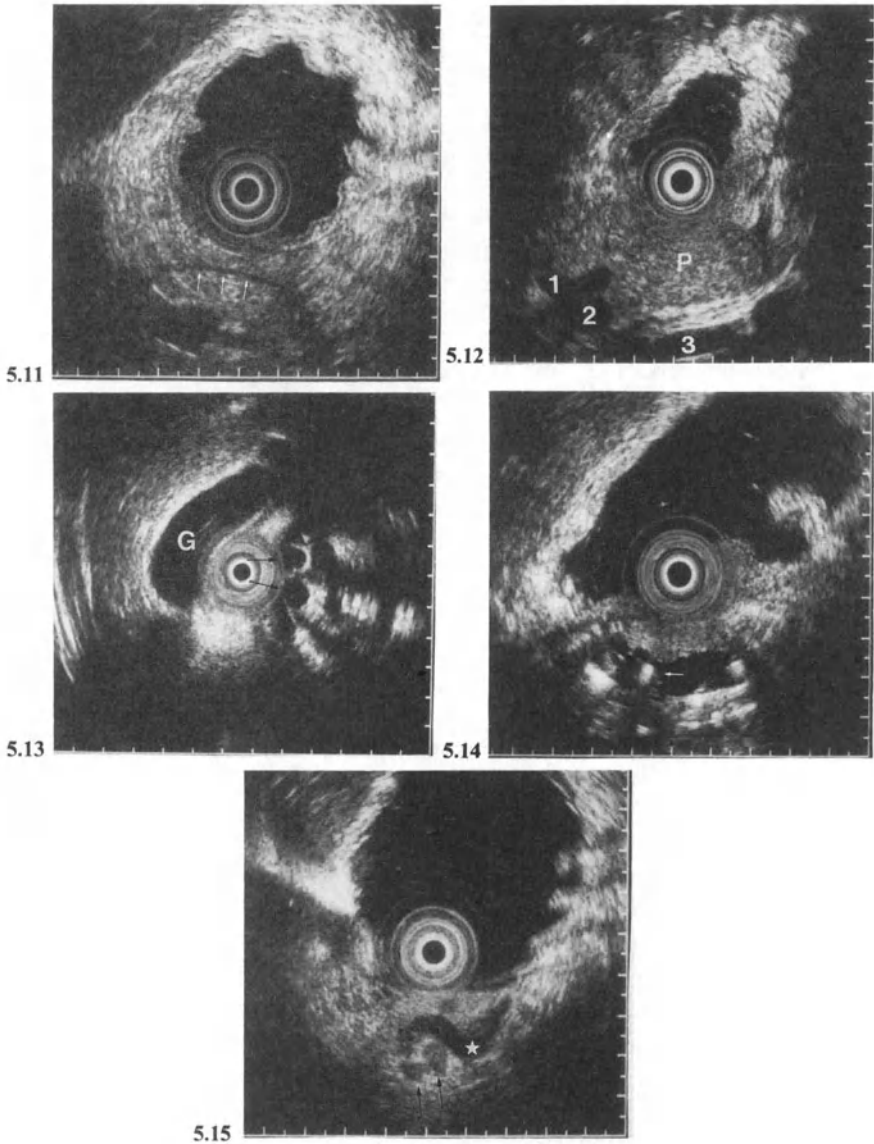


Fig. 5.11 Pancreatic body with normal main pancreatic duct (arrows)

Fig. 5.12 Tail of the pancreas (P): 1 = splenic artery; 2 = splenic vein; 3 = left renal vein

Fig. 5.13 Small pancreatic pseudocysts (arrows): G = gall bladder

Fig. 5.14 Dilated main pancreatic duct. Note the irregularities of its wall. Arrow = intraductal stone

Fig. 5.15 Metastatic lymph nodes (arrows) around coeliac trunk (asterisk)

Table 5.2 Endoscopic sonography in oesophageal diseases ($n = 32$)

Carcinoma	19 (complete passage possible in 7)
Lymphoma	1
Achalasia	6
Submucous tumour	2
Barrett's oesophagus	4

COMPLICATIONS

Complications of endosonographic examinations of the upper GI tract have not been reported, and we did not experience any. Nevertheless sedation of the patient, especially in older persons with chronic obstructive lung disease and cardiocirculatory disorders, can lead to side-effects such as cardiac arrhythmias, a fall in blood pressure and even to respiratory arrest. The necessity to fill the stomach with water carries the risk of an aspiration pneumonia. To diminish this risk we do not perform pharyngeal anaesthesia. Since sonographic scopes are still more rigid than conventional gastroscopes, and are equipped with a side-viewing optic, one must be very careful, especially in the oesophagus, to avoid mucosal lesions or even perforations of fragile carcinomatous tissue. In these cases a radiological barium swallow examination prior to endosonography is recommended, to detect the location and extent of a stricture.

EFFICIENCY OF, AND INDICATIONS FOR, ENDOSCOPIC ULTRASOUND

Before a new method can be introduced into clinical practice its value has to be compared to already existing methods, and its efficacy has to be shown in defined clinical settings. Many publications regarding endoscopic ultrasound deal with the diagnostic potential of this method. It should therefore be welcome that, especially in the recent past, controlled studies have been performed to evaluate the diagnostic efficiency of this technique.

The diagnosis of *oesophageal carcinoma* has been made correctly by Heyder *et al.*⁹ in 27 out of 30 cases. We have performed endosonography studies on 32 patients with diseases of the oesophagus (Table 5.2)¹⁰. Nineteen patients had histologically proven carcinoma

of the oesophagus and the diagnosis was known beforehand to the investigator. Endosonography was performed to assess the pre-operative staging capability of the method. Based on the sonographic criteria mentioned above, the carcinoma could be demonstrated endosonographically in all cases. However, passage of tumour stricture was possible only in seven of the 19 cases. Benign submucous tumours could be seen in two cases, and in six patients with stenosis due to achalasia a tumour was excluded endosonographically after pneumodilatation in five patients. Four patients with Barrett's oesophagus are regularly followed up by endosonography. Although in one case cytological brushings led to the suspicion of a malignant tumour, on endosonography the entire wall of the oesophagus was smooth. The patient was not operated on, and the clinical course in this case confirmed the endosonographic diagnosis. In a second case cytologically dysplastic cells were described. On endosonography a localized thickening of the oesophageal wall was demonstrated, and was confirmed at operation to be a carcinoma. In the follow-up of patients with an endobrachyoesophagus endosonography represents a valuable additional tool.

Although in cases of known oesophageal carcinoma the nature of the stenosis was assessed correctly, preoperative staging of the carcinoma by endosonography at the present time is limited, since tumour stricture cannot be passed in the majority of cases, and thus it is impossible to evaluate tumour tissue beyond the stenosis. These results, however, should not be discouraging, since they are only partly due to the method itself. They rather mirror the clinical problem that, in most patients seen by us, the oesophageal carcinoma was already far progressed. Controlled studies by Japanese authors¹¹ demonstrate that endosonography at the present time is the most sensitive tool in the delineation of the extension of small oesophageal tumours. When early diagnosis is made, excellent long-term results will be obtained. It is well known that oesophageal cancer spreads both longitudinally and circumferentially in the oesophageal wall. Submucosal spread is beyond endoscopic visibility, and although CT and magnetic resonance imaging have made staging before operation more accurate, demonstration of submucosal spread often is missed by these methods. The logical consequence of our results therefore seems to be to apply endosonography early in the diagnostic evaluation of dysphagia. In

our hospital each dysphagia whose aetiology is not clarified completely by endoscopy, radiology or neurological examination represents an indication for endosonographic evaluation.

The possibility of determining the depth of infiltration of *gastric tumours* justifies the hope that endosonography will also play an important role in this clinical setting¹². First reports dealing with the diagnostic value of endosonography in early gastric cancer are promising. Takemoto's group even treats the endosonographically verified early carcinoma without metastases with endoscopic laser. At the present time, however, a final judgement in this regard is not possible.

The demonstration of extragastric lesions that impress the gastric wall will also be a domain of endosonography, although large controlled studies regarding this issue are not available. The delineation of the pancreatic body and tail succeeds regularly. Since, however, with the presently available instruments the pyloric passage is only possible in about 80% of cases a systematic approach to the head of the pancreas and to the papilla Vateri is not possible regularly. Our investigations¹³ of the efficiency of endosonographic diagnosis of *benign pancreatic and biliary lesions* demonstrate that of 87 examined patients 12 had a chronic calcifying pancreatitis, eight of whom also had pseudocysts. In all 12 cases the diagnosis could be made correctly with endosonography, and in addition it was possible to demonstrate even small pseudocysts, diameter was less than 0.5 cm, which were not seen by CT or sonography. Even small exudates in the omental bursa not visualized by other methods were visible endosonographically. The clinical importance of such a finding remains to be defined. Eighteen of our patients had a dilated common bile duct that could be seen, at least partially, endosonographically, and in seven out of eight cases stones in the main bile duct were demonstrated endosonographically. A histologically proven adenomyosis of Vater's papilla escaped endosonographic demonstration in three patients. We examined three cases with the clinical manifestations of *hormone-producing pancreatic tumours*. An insulinoma, 1.5 cm in diameter, and a large glucagonoma in the pancreatic tail were seen by endosonography. These tumours were also demonstrable with conventional methods. A small insulinoma (0.5 cm diameter) in the dorsal part of the pancreatic head could not be seen on endosonography or with

conventional ultrasound, computed tomography, angiography or nuclear magnetic resonance. The tumour was finally localized during operation by the palpating finger of the surgeon. The experiences of other investigators¹⁴ show, however, that thanks to the excellent detailed resolution of endosonography even small APUDomas are demonstrable. Yet the total number of cases reported in the literature is too small to permit a final judgement at the present time.

In 118 patients examined by endosonography Yasuda *et al.*¹⁵ found a *pancreatic carcinoma* in 13 cases. Nine of these tumours were larger than 3 cm in diameter, one had a diameter of 2.5 cm, and in the remaining three cases the tumour was smaller than 2 cm. All these tumours were echo-poor. In 10 of these 13 cases, besides conventional and endoscopic ultrasound ERCP, computed tomography and angiography were also performed. The positive finding rate of endosonography reported by the authors was 100%, for conventional ultrasound 40%, for ERCP 80%, computed tomography 70% and for angiography 80%. None of the three tumours smaller than 2 cm showed any metastases and could be resected curatively. From these studies the authors conclude that, compared to ERCP, CT, angiography and conventional ultrasound, 'endosonography is the most useful method for the demonstration of small pancreatic carcinomas of less than 20 mm size'. In a further prospective study¹⁶ aimed at investigating the sensitivity and specificity of endosonography compared to conventional morphological methods in the diagnosis of pancreatic cancer, 34 patients with painless jaundice were examined. Endosonography was performed after conventional ultrasound. Three tumours of Vater's papilla were detected by endosonography, and conventional ultrasound demonstrated only one of them. Ten pancreatic cancers with a mean size of 2.8 cm were diagnosed by endosonography. According to the authors endosonography permitted a more precise description of outer tumour margins and of its inner echo structure compared to conventional ultrasound, so that in addition to the demonstration of a pathological lesion endosonographic criteria allowed better discrimination between benign and malignant alterations. Six of their cases were echo-poor, two echo-dense and two showed a mixed reflex pattern. It was remarkable that four malignant tumours showed smooth outer contours. Both studies cited are laudable efforts to assess objectively the diagnostic efficiency of endo-

sonography in the diagnosis of pancreatic cancer. The endosonographic demonstration of a large tumour that has already been visualized by other methods is, of course, of no prognostic advantage to the patient. An interesting starting point for further prospective studies that must aim at the endosonographic diagnosis of potentially curatively resectable tumours is the ability of the method to demonstrate small tumours that escape delineation with conventional methods¹⁷. However, due to the natural course of pancreatic carcinoma a systematic early detection of this neoplasia with endoscopic ultrasound is not to be expected. In this case, too, endosonography is to be viewed as a complementary method.

The excellent capability of conventional ultrasound in the diagnosis of *cholecystolithiasis* makes an endosonographic examination in these cases superfluous. Since, however, the gall bladder is frequently visualized during examinations performed for other reasons the high detail resolution of this method will again be evident. The same is true for the *liver*, that is clearly seen with conventional ultrasound, and therefore it is not to be expected that endosonography will play an important role in the diagnosis of liver diseases. Nevertheless it is worthwhile to perform endosonographic study of tumours of the bile ducts that are localized in the hilum of the liver, or spread in the hepatoduodenal ligament and infiltrate the porta hepatis. Clinical experience shows that these tumours are not visualized by sonography, computed tomography, angiography or nuclear magnetic resonance, although they might have already attained a large size. Stenosis, and proximal dilatation of the bile ducts, are frequently the only indirect tumour signs, and a short stenosis is often caused by a tumour that proves to be irresectable. It would mean tangible progress if it became possible, with endosonography, to delineate tumour size accurately before operation.

CONCLUSIONS AND A LOOK TO THE FUTURE

From the preceding sections it should have become clear that endoscopic sonography of upper GI tract in most clinical settings is still to be regarded as a clinical experimental method. The indications for an endosonographic examination should be restricted (Table 5.3). They must be deduced from the technical features of the method, namely

Table 5.3 Indications for endoscopic sonography of the upper GI tract

Oesophagus	Dysphagia Barrett's oesophagus Achalasia (after dilatation) Submucous tumours Carcinoma
Stomach	Intraluminal and submucous tumours Extragastric impressions Carcinoma Lymphoma Ménétrier's gastritis 'Prominent folds' of unknown etiology
Pancreas	Search for small (< 2 cm) carcinoma Tumours of Vater's papilla Hormone-producing tumours

high detail resolution and low penetration of the ultrasonic beam. We expect that endosonography will be helpful in planning palliative laser treatment and radiation-afterloading therapy of oesophageal cancers by accurately describing the depth of tumour invasion. Additional information will also be gained from endosonography in portal hypertension. Thus potentially deleterious snare biopsies of prominent folds seen on gastroscopy will be avoided by a prior endosonographic examination that shows such folds to be made up of intramural varicose vessels.

The procedure is not to be regarded as an alternative to conventional ultrasound, and endosonography should only be performed to assess the wall of the GI tract itself, or tissues and organs in its immediate proximity. Since the procedure requires a fair amount of endoscopic and sonographic skill the method should only be performed by physicians well trained in these techniques. Complications have not been reported yet, but due to the premedication should be kept in mind, especially in older patients with cardiac and pulmonary diseases. Filling the stomach with water carries the risk of an aspiration pneumonia.

Unquestionably marked technical progress has been achieved in recent years, and technical developments will lead to further noticeable

improvements in endoscopic ultrasound. The aim must be to reduce the diameter of the scopes, to make the instruments more flexible and, in addition to side-viewing instruments, forward-viewing endoscopes equipped with ultrasonic probes should be introduced. The latter should provide a sector scan image even if this leads to a modest reduction of the 360° view. The presently available mechanical scanners should be replaced by electronic devices. We assume that in future the effort to perform an endosonographic examination will be comparable to a 'simple' gastroscopy with the opportunity to obtain tissue biopsies, to perform transmural fine-needle biopsies and to switch on the endosonographic transducer as required. These major breakthroughs will lead to an increase in the use of endosonography in clinical routine.

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