# REFLUX OESOPHAGITIS

## An experimental study in rats

**PROEFSCHRIFT**

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# A. GENERAL PART

CHAPTER I

## INTRODUCTION

The aim of the surgical therapy of reflux oesophagitis is prevention of reflux, and the treatment of the irreversible complications of reflux such as strictures. Recent developments in the treatment of reflux are concentrated on an earlier diagnosis of reflux and oesophagitis and prevention of complications.

The discussion as to the best surgical treatment of reflux, has in the course of years suggested several types of operations:

- Thoracic reconstruction of oesophageal hiatus according to Allison (1951).
- Anterior gastropexy according to Boerema (1955).
- Abdominal fundiplication according to Nissen (1959).
- Trans-thoracic fundiplication (Mark IV) according to Belsey (1967).
- Posterior gastropexy according to Hill (1967).

The indications for operative treatment of reflux oesophagitis differ from one centre to another. Generally speaking only patients with reflux, and symptoms and signs of reflux oesophagitis are treated.

The results of the surgical techniques mentioned above, vary considerably (DeMeester et al, 1974, Behar, 1979). They give little insight in the pathogenesis of the oesophageal inflammation which can occur in the presence of reflux. In the approach to this problem, one could be satisfied with the development of an effective antireflux operation, but perhaps it is more realistic first to study more in detail the fundamental causes of oesophagitis. This then may offer arguments leading to a more rational indication and choice of operative treatment.

At present there are many contrasting theories concerning the etiology of reflux oesophagitis in man. Experimental data is also contradictory.

The two most important current theories are:

1. *The acid-pepsin theory.*

   This theory, proposed as far back as 1879 by Quincke, suggests that it is gastric juice that produces oesophagitis when gastro-oesophageal reflux is

present (Allison, 1948, Atkinson and Van Gelder, 1977). However, oesophagitis has been shown to occur in patients with achloorhydria (Orlando and Bozymski, 1973) and also in patients who have undergone total gastric resection (Helsingen, 1960). Furthermore the fact that inhibition of acid and pepsin production by a drug such as cimetidine, does not always result in cure of reflux oesophagitis, tend to discredit the acid-pepsin theory (Powel Jackson et al, 1978, Behar et al, 1978, Wesdorp et al, 1978, Ferguson et al, 1979).

2. *Biliary reflux theory.*
   According to this theory, bile is at least partly responsible for the existance of reflux oesophagitis. Both clinical and experimental work (Gillison et al, 1972, Henderson et al, 1973, Crumplin et al, 1974) seem to support this theory. At present there is no conclusive proof that bile induces oesophagitis when gastro-oesophageal reflux is present.

On basis of these two theories, many surgical clinics are treating severe stenosing reflux oesophagitis with both and acid reducing operation (e.g. vagotomy and antrectomy), and a (Roux en Y) biliary diversion procedure. In general the results are so favourable that the outcome of this combined procedure seemed to support the hypothesis that gastric juice and bile are probably together responsible for the presence of reflux oesophagitis (Royston et al, 1975). When stenosis has already developed, whether the oesophagus is shortened or not (Ellis and Leonardi, 1978), in a large number of patients frequent dilatation is required.
Furthermore, sometimes an oesophagus lengthening procedure is even required to restore a good passageway in addition to acid reducing and biliary diversion procedures.

The idea that oesophagitis can develop only when gastro-oesophageal reflux is present, is at present generally accepted. In the search for factors causing oesophagitis, much importance has been placed upon the hiatal oesophageal hernia. Although the correlation between hiatal oesophageal hernia with reflux and the development of oesophagitis is not clear, only about half of the patients with hiatal oesophageal hernia are symptomatic (Dunphy, 1979). These symptoms are thought to be produced, mainly by the concomitantly present oesophagitis. Furthermore, the fact that not all patients with gastro-oesophageal reflux develop oesophagitis suggests, that not reflux as such, but the factors in the reflucting juice may play the most important etiological role (DeMeester et al, 1976).
For this reason this study concentrates mainly on these aspects of reflux. As mentioned before, gastric juice and bile have been studied rather extensively.

In experimental studies pancreatic juice has got some attention (Cross and Wangenstein, 1951, Lambert, 1962). One has however to consider that although the authors speak of pancreatic juice, infact what they tested was duodenal juice without bile.
We decided in agreement with these authors to use also (for practical reasons) the term pancreatic juice, in stead of duodenal juice without bile, as pancreatic juice is probably the most important substance of the duodenal content besides bile.

One of the problems in the clinical diagnosis of reflux oesophagitis, is the discrepancy sometimes found between the endoscopical appearance of the oesophageal mucosa, and the histology of the biopsies taken. However, histological examination of biopsy material of such "normal" mucosae reveals, in a large percentage of cases (78%) definite abnormalities, i.e. increase in papillary height, acanthosis, basal cell hyperplasia and acute inflammatory infiltrate in the lamina propria (Ismail-Beigi et al, 1970). These signs are accepted by many histologists and clinicians to be the result of "irritation" of the oesophageal mucosa by gastro-oesophageal reflux, and thus representing an early and/or mild form of reflux oesophagitis. This problem will get special further attention in the study presented in this thesis.

Many attempts at producing oesophagitis experimentally have been made. Different results have been observed with the same stimuli, among the same, as well as among different species of laboratory animals. Opinions about the etiology of oesophagitis are thus rather divergent. Furthermore neither these experimental studies nor studies on human reflux oesophagitis give a good insight into the kinetics of reflux oesophagitis.
Studies on oesophagitis in man are dependant upon the histology of oesophageal biopsies. Here only an impression of a part of the oesophageal wall is obtainable.
Histological studies of autopsy specimen are often hampered by autolysis. In animal experiments, on the other hand, a more complete insight into abnormalities taking place in the full thickness of the oesophageal wall can be obtained. This may lead to a better understanding of the different phases of oesophagitis occuring with the course of time.

The rat oesophageal mucosa differs with the oesophageal mucosa in man, in that it has keratinised epithelium, and the musculature in striated. In view of the uncertainty of the etiological factors of oesophagitis and its development in time, a study in the rat seemed however justified, because the presence and course of oesophagitis can be studied at different time intervals.

The rat was also chosen because reliable statistical analysis could be performed on the experimental results. This may give much needed insight into the kinetics of the inflammation which is still missing in the literature, especially where fibrosis is concerned.

The objectives which formed the basis of the studies presented in this thesis were:

1. To find whether reflux oesophagitis arises from gastric juice, pancreatic juice, bile, or a combination of these secretions.
2. To find, in the case of reflux oesophagitis, how the inflammation progresses.
3. To determine which part of the oesophagus is most strongly involved.
4. To determine which changes can be macroscopically, as well as microscopically recognized, and to find to what extent a correlation exists between these two.
5. To find, if fibrosis does appear, when it starts and whether or not is leads to stenosis (seen in relation to time and stimulating secretion).
6. To find whether or not fibrosis is moreover the forerunner of shortening of the oesophagus.
7. To asses whether metaplastic changes and/or atypical cells occur during the course of the experimentally induced oesophagitis.
8. To determine if duodenal diversion, effective in man in preventing oesophagitis, is as effective in this experimental model.

CHAPTER II

# SURVEY OF THE LITERATURE

## 1. Gastric acid, pepsin and reflux oesophagitis.

Quincke described oesophageal ulcers in three patients as early as 1879. The conclusion he drew was that these ulcers were caused by reflux of gastric juice (ulcer oesophagi ex digestion). This was mainly because of the anatomical localisation of the ulcers, distal in the oesophagus and their macroscopical similarity to stomach ulcers.
Years later Jackson suggested (1925) that upper respiratory tract infection was responsible for the development of oesophageal ulcers.
In 1935 Winkelstein analysed the then prevailing ideas of possible causes of oesophagitis. Chronic irritation by chemical, thermic, or mechanical factors was considered to cause oesophagitis. Specific infections (Lues, tbc, actinomycosis) were also incriminated. Finally it was suggested that oesophagitis could arise secondary to cardiospasm, diverticula or neoplasma in the oesophagus. The fact that many patients with oesophagitis did not fall into the above mentioned groups, brought Winkelstein (1935) to the conclusion that most cases of reflux oesophagitis seemed to have a "peptic" origin.
In 1938 Seleye, referring to a post mortem study of patients with hemorrhagic ulcerous changes in the oesophagus, concluded that the changes were due to a "peptic digestion" rather than autolysis.
In that very year Allison (1948) also put forward the view that reflux oesophagitis had a "peptic" origin. He described the symptoms and radiologic findings of 74 patients with oesophageal ulcers. He postulated reflux of gastric juice due to insufficiency of the cardia and in this way "peptic" oesophagitis could be explained. He described at the same time four patients who developed a "peptic" oesophagitis after resection of the cardia (for carcinoma of the cardia) and oesophageal reconstruction e.g. oesophago-gastrostomy. In the fifties the acid pepsin theory received even more following, as the only and most important factor in the pathogenesis of reflux oesophagitis in patients with a sliding hiatal hernia.

In 1965 Ellis and Hood published favourable results from operations on 11 patients with so called "peptic" strictures of the distal oesophagus; in these patients the distal oesophagus and cardia were excised and at the same time a resection of the distal portion of the stomach and a vagotomy were carried out to reduce acid production. Other arguments which could point towards acid and pepsin as being the causative factors for reflux oesophagitis, are the fact that in some studies the oesophagitis is often associated with an increased acid secretion, and the fact that concomitant ulcers are also often found. Burge et al (1966) and Casten (1967) found duodenal ulceration in 50% of the patients with so called "peptic" oesophagitis.
This was however contrary to the findings of Brain (1966) who found that only 4,2% of patients with reflux oesophagitis had concomitant duodenal ulcerations.
Stol and Collis (1974) who likewise sought a correlation between acid secretion and the occurence of oesophagitis, found a definite rise in basal acid secretion in patients with a "symptomatic" hiatal hernia, especially in the presence of oesophagitis. Slight increase in maximal acid secretion was also found in "symptomatic" hiatal hernia patients with and without oesophagitis. On the basis of these findings the idea that also non surgical reduction of acid production with recently produced acid and pepsin reducing drugs, seemed promising (Wesdorp et al, 1978).
However, Behar et al (1978) and Powel-Jackson et al (1978) found no histological improvement of reflux oesophagitis in patients who used 1600 mgr cimetidine daily during 6-8 months. Ferguson et al (1979) also found no improvement of oesophageal stricture and histophathological changes of the mucous membrane in 20 patients treated 6 months with 1600 mgr cimetidine daily.
Until now the data related to the acid-pepsin theory in the human has been quoted. Below experimental data are given. For the sake of clarity it is given according to the different species of laboratory animals on which the experiments were performed.

*The rat.*

The first experiments on rats were described by Seleye (1938). He found that ligation of the pyloris in rats resulted in a serious hemorrhagic lesion in the lower thoracic portion of the oesophagus. He found no lesions in the abdominal, the remaining thoracic or cervical portion of the oesophagus. One of the 6 rats even suffered oesophageal perforation. The pylorus and cardia were ligated in further group of six rats. Here no oesophageal lesions

were found. Seleye (1938) concluded that these lesions were the result not of autolysis but of so called "peptic" digestion of the oesophageal mucosa.
Lodge (1954) performed experiments on rats (n = 60) in which the stomach content was acidified with 0,5% NaCL. After 24 days, 87% of the rats had ulcers in the rumen of the stomach and 23% had also some ulcers in the distal portion of the oesophagus. By addition of $NaHCO_3$, the pH in the stomach was elevated and no ulcer formation occured. By excising 1 cm of the muscle cuff of the distal oesophagus in another group of rats, gastric reflux was achieved. This alone brought about oesophageal ulceration. If vagotomy was performed in addition, ulceration was prevented. However, administration of acidified food seemed to result in the reappearance of distal oesophageal lesions.
However, Lambert (1962) found in sharp contrast to findings of Seleye (1938) and Lodge (1954), that reflux of gastric juice alone did not result in oesophagitis.

*The dog.*

The main support for the "acid-pepsin" theory is found in studies performed on the dog. Many of these were done in the fifties.
Ellis and Hood in 1954 published a paper describing gastric reflux in dogs achieved by resection of the cardia with an end to end reconstruction; this procedure induced an ulcerative oesophagitis. By performing an additional resection of the fundus and corpus of the stomach (so removing whole the acid secreting portion of the stomach) the development of significant degrees of oesophagitis was prevented. They did not, however, perform histological examination of the oesophageal mucous membrane and/or assess for duodenal reflux.
Kiriluk and Merandino (1954) performed an experiment on dogs in which the mucosa of various parts of the gastro-intestinal tract was exposed in vivo to a solution of 0.1 N.HCL and 1% pepsin. The duration of the drip varied from 15 minutes to fifteen hours. The animals were sacrificed at one, two, three and fifteen days after the dripping procedure. Gastric mucosa appeared very resistant. That of the duodenum, the jejunum and the ileum appeared vulnerable. The lesions, however, healed rapidly when the "perfusion" was stopped. The oesophageal mucosa too, was susceptible to ulceration, and at the same time was slow to heal. Increase of the pepsin concentration of the perfusate did not enhance the caustic effect.
In 1958 Adler et al performed experiments to determine whether or not there was a difference in resistence to a solution of HCL and pepsin between the

upper and lower oesophagus. According to clinical observations oesophagitis seldom occured after extensive oesophagus resection with a high oesophago-gastrostomy. Using in vivo dog studies, a solution of 0.1 N.HCL and 1% pepsin was allowed to drip at different levels in the oesophagus. Autopsy was performed at one, two, four, seven and fourteen days post-operatively. Ulcerative oesophageal lesions arose after 48 hours at all levels of the oesophagus tested.

In 1958 Redo and Barnes studies the effects of reflux of gastric juice, of duodenal juice together with gastric juice, of jejunal and of colonic juice on the dog oesophagus. The reflux was achieved by anastomosing the oesophagus (end to side) with a portion of stomach, of duodenum, of stomach and duodenum and of jejunum or colon through the diaphragm. Gastric juice gave rise to ulcerations. Gastric juice together with duodenal juice brought about linear erosions. Duodenal juice alone gave superficial erosions. No reaction was found when jejunum or colonic fluid entered the oesophagus. Redo et al (1958) also performed a "perfusion" experiment to study the reaction of oesophageal mucosa of the dog to 2% artificial pepsin solution, 0.1 NHCl, human bile together with human pancreatic juice and a mixture of human bile together with gastric juice from other dogs. The "perfusions" lasted from 3 to 8 hours. At the end of the experiment the dogs were sacrificed. A solution of 2% pepsin with pH $<$ 2 proved strongly ulcerative. Human bile, and also 0.1 N.HCl alone produced only edema of the oesophageal mucous membrane. Human bile together with human pancreatic juice produced slight erosion. Human bile with gastric juice produced slight erosion at pH $<$ 2.4. When the pH of this mixture was greater than 2.4 no erosion occured.

Barnes et al (1968) performed studies on dogs in which 2% pepsin solution with pH $<$ 2 (during 3½ - 7 hours) was allowed to drip through the oesophagus. Severe ulceration occured under these circumstances and at the same time "this perfusion" produced sporacic appearance of perforation. The most extensive lesions arose in the segment closest where the solution was allowed to drip.

*Summary.*

According to studies on oesophagitis in men, gastric juice seems to be important for the production of reflux oesophagitis, however, it is not clear if in these studies one always can speak of reflux of true gastric juice alone. A mixture of gastric and duodenal juice could also be involved! Furthermore there are strong arguments against the HCl-pepsin theory as the sole factor

causing reflux oesophagitis i.e. the appearance of reflux oesophagitis in achlorhydric patients (Palmer, 1960, Orlando and Bozymski, 1973) and the fact that cimetidine a potent inhibitor of the secretion of gastric acid and pepsin, does not always result in cure of oesophagitis in patients with gastro-oesophageal reflux. Interestingly enough the symptoms of oesophagitis such as heartburn decrease with treatment with cimetidine. Rat experimental findings appear contradictory. According to studies by Seleye (1938) and Lodge (1954) in the rat, reflux of gastric juice give rise to oesophagitis. This was clearly not the finding in experiments of Lambert (1962) and Levrat et al (1962). Data in the literature about oesophagitis in dogs appear to verify that HCl-pepsin is responsible for the origin of reflux oesophagitis, although the refluctingjuice in some experiments could have been a mixture of duodenal and gastric juice rather than gastric juice alone. Furthermore many of these experiments are non physiological, e.g. "drip" experiments, in which variable amounts of concentrated solutions of pepsin or HCl and HCl and pepsin together are dripped locally. Also the duration of the "perfusion" times varied considerably.

**2. Bile, other duodenal factors and reflux oesophagitis.**

Tending to descredit the acid-pepsin theory are the facts firstly that patients with little or no acid production, can still develop oesophagitis in the presence of gastro-oesophageal reflux (Palmer, 1960, Orlando and Bozymski 1973), and secondly that oesophagitis has been observed in patients who have undergone a total gastric resection. This was particularly noted if the continuity was restored with an oesophago-duodenostomy as described in the clinical study of Helsingen (1960). Helsingen therefore suggests that duodenal factors are of main importance in the etiology of reflux oesophagitis. After this, many more studies were carried out to test the hypothesis that a factor (or factors) produced in the duodenum is (are) partially or fully responsible for the occurence of reflux oesophagitis.
In 1961 Holt and Large studied patients with reflux oesophagitis who had had a cardioplasty for cardiopasm. The oesophagitis of these patients persisted, in spite of an additional subtotal gastric resection. Analysis of the fluid aspirated from the inflamed oesophagus at various levels in these patients, showed all samples to contain high concentrations of bile and amylase. On the basis of this observation they concluded that not only the regurgitation of gastric juice, but also that of duodenal content could produce oesophagitis. In order to prevent this, these workers performed a duodenal diversion by a Roux en Y procedure in addition to the cardioplasy,

and distal subtotal gastric resection. It is to be noted that more importance was placed on bile than the other factors in the reflucting duodenal juice. Eleven patients reexaminated eight years later, showed excellent results.
In 1974 Stol and Collis found significally high total bile acid (TBA) concentrations and a significantly elevated basal acid output in the stomachs of patients with a sliding hiatal hernia and symptomatic reflux. The pathological lesions found in the oesophagus appeared to correlate with the increased total bile acid concentration in the stomach, and with the increased basal acid output.
Crumplin et al (1974) also found an increase in total bile salt concentration and a raised basal acid secretion in stomachs of patients with a "symptomatic" sliding hiatal hernia and reflux oesophagitis. This implies pyloric insufficiency in these patients. Crumplin emphasized that the combination of gastric juice and bile played a major role in the pathogenesis of reflux oesophagitis. Many animal experiments have been performed to study the possible role played by duodenal factor(s) in production of oesophagitis. Those in the rat, dog, cat and monkey will be described successively.

*The rat.*

As oesophagitis could occur in patients who had undergone total gastric resection with continuity restored by oesophago-duodenostomy, Helsingen (1960) decided to perform the same operation on rats. These rats also developed an extensive oesophagitis. This was regarded as support for the hypothesis that duodenal juice plays the more important role in procuding reflux oesophagitis than factors in gastric juice. The same year he carried out a further experiment. This time he performed a total gastric resection, restored continuity with an oesophago-duodenostomy or an oesophago-jejunostomy, with and without a Brauns anastomosis or a Roux en Y procedure. If continuity was restored with oesophago-duodenostomy, extensive lesions resulted. In the case of an oesophago-jejunostomy, especially if this was combined with a Brauns anastomosis, a much milder oesophagitis was found. If continuity was restored with an oesophago-jejunostomy and a Roux en Y jejuno-jejunostomy, the degree of severity of oesophagitis could be influenced by varying the length of the Roux en Y afferent jejunum loop. The longer the Roux en Y loop, the less the oesophagitis. Indeed if this Roux en Y loop was of sufficient length, no oesophagitis resulted.
In 1962 Lambert performed an oesophago-jejunostomy with or without

biliary diversion by cannulating the bile duct and/or total gastric resection. In 79% of the rats severe oesophagitis was seen with reflux of gastric juice and duodenal juice together. Only 28% of the animals in which reflux of bile only was induced, suffered an oesophagitis. Lambert concluded that pancreatic juice was one of the main factors responsible for the occurence of oesophagitis.
In 1962 practically the same experiment was performed by Levrat et al, where selective reflux of pancreatic juice, gastric juice, or combination of gastric juice, bile and pancreatic juice was achieved. Bile together with pancreatic juice produced the most severe lesions. With reflux of gastric juice alone no lesions arose.

*The dog.*

In 1951 Cross and Wangenstein performed an experiment on four groups of dogs in which, an end to side duodeno-oesophagostomy, a cholecystojejuno-oesophagostomy, a jejuno-oesophagostomy or a total gastric resection with end to end oesophago-duodenostomy was carried out. These procedures resulted in reflux of pancreatic juice and bile, bile only, and intestinal juice respectively. Autopsy was performed between 1-3 months later. After 8 days, bleeding erosions on the oesophageal mucosa occured with reflux of bile and/or pancreatic juice. The oesophageal lesions became more extensive with the passage of time. With reflux of intestinal juice no lesions arose.
Moffat and Berkas (1965) achieved biliary reflux in six dogs by anastomosing an intrathoracic isolated jejunal loop to the distal portion of the oesophagus (end to side). Bile was diverted into this segment after ligation and division of the common bile duct distal to the cystic duct, and anastomosing the gallbladder tot he jejunal loop. The dogs were sacrificed from 2 weeks to 5 months postoperatively. In all dogs a severe erosive type of oesophagitis resulted. This was evident on oesophagoscopy by the second week. In another group of 10 dogs an intrathoracic isolated jejunal loop was anatomosed to the distal part of the oesophagus for chronic biliary injections. Solution of bile with pH-8, bile with pH 2-4, bile with pH 10-12 and bile salts was allowed to run through this jejunal loop. These solutions (100 cc) were injected in doses of 25 cc four times daily. Oesophageal erosions were seen on oesophagoscopy within three to six weeks. The destructive effect of these solutions was independent of pH.
In 1973 Henderson et al performed a "perfusion" experiment with bile salts, 0.1 N.HCl, 0.1 NHCl together with bile, and 0.1 N.HCl mixed with bile salts (taurocholate or glycocholate). The solutions (100 cc) were dripped on the

oesophageal mucous membrane over a four hour period daily, for 21 days. Results showed that perfusion with bile, bile salts or 0.1 NHCl resulted only in a mild oesophagitis. When 0.1 NHCl was mixed with bile, glycocholate or taurocholate, severe oesophagitis resulted. According to these workers, combination of 0.1 N.HCl and taurocholate proved the most damaging.

*The cat.*

Cross en Wangenstein (1951) performed an experiment on cats, where under nembutal anaesthesia the oesophagus was "perfused" with bile and pancreatic juice, bile only, pancreatic juice only, and dog intestinal fluid. Human bile, and bile salts of different concentration were also used in this experiment. The duration of "perfusion" was about 8 hours. After this "perfusion" the cats were sacrificed. Severe lesions were seen to arise with perfusate of pancreatic juice and bile together. "Perfusion" with human bile and with sodium taurocholate and sodium glycocholate also produced an oesophagitis. Pancreatic juice alone caused only minimal changes, whereas dog intestinal fluid produced no change of the oesophageal mucous membrane.

*The monkey.*

Gillison et al (1972) performed experiments on monkeys, where reflux of gastric juice, and gastric juice contaminated with bile were compared. The cardia was excised to eliminate the sphincter mechanism. The vagal nerves, however, were left in tact. In one group the galbladder was anastomosed with the stomach. The animals were sacrificed from 3-6 months postoperatively. These workers found that reflux oesophagitis arose sporadically with gastric reflux alone. With bile contaminated gastric juice, however, every form of oesophagitis arose.

*Summary.*

Some data from the literature about oesophagitis in humans point to one or more duodenal factors as being of essential importance in the development of human oesophagitis, whether or not in combination with gastric juice. Bile in particular was extensively studied. Experiments performed on rats, seem to indicate that bile together with pancreatic juice is necessary for the occurance of reflux oesophagitis. There is a lack of uniformity of results in experiments performed on the dog.
According to Cross and Wangenstein (1951) both bile and pancreatic juice

can procude oesophagitis in dogs. Contrary to this, Redo et al (1959) saw practically no oesophagitis with bile, or a combination of bile and pancreatic juice, in his "perfusion" experiment. This experiment was, however, a non physiological "drip" experiment and of short duration. In an experiment performed on cats (Cross and Wangenstein, 1951), severe ulcerating oesophageal lesions resulted from pancreatic juice and bile in combination or from bile and from bile salts alone. This was, however, also a "drip" experiment.
In Gillisons (1972) study on monkeys, gastric juice contaminated with bile particularly seemed to be responsible for the presence of oesophagitis. However, the presence of other duodenal factor(s) was not excluded i.e. adequacy of the pyloric sphincter was not confirmed.

### 3. Roux en Y oesophago-jejunostomy and reflux oesophagitis.

Support for the hypothesis that one or more duodenal factors is(are) responsible (or partly responsible) for the occurence of oesophagitis, can be found in clinical and experimental studies where the Roux en Y oesophago-jejunostomy procedure is used, either to treat or to prevent the oesophagitis.
After total gastric resection in rats, Helsingen (1960) restored continuity in a variety of ways. When continuity was restored with an oesophago-duodenostomy, oesophagitis developed. If Roux en Y oesophago-jejunostomy of appropriate length was constructed, oesophagitis could be prevented.
Gillison performed a study on the monkey (1972), that also showed that oesophagitis could be prevented by a Roux en Y oesophago-jejunostomy.
In 1956 Scott and Weidner described a serie of patients (n = 23) with gastric carcinoma, who underwent total gastric resection with Roux en Y reconstruction. During 5 year follow up of these patients, no reflux oesophagitis occured.
Balint and Gummer (1958) described similar results in a series of 12 patients who received the same operation for recurrant ulceration on the anastomosis after previous partial gastrectomy and vagotomy.
Weaver et al (1970) described a group of patients with severe reflux oesophagitis, who in 8-17 years follow-up, showed definite improvement after Roux en Y gastro-jejunostomy.
In 1971 Mackman et al described a series of patients with "biliary gastritis" and "biliary oesophagitis". A Roux en Y procedure lead to cure in alle patients.
Royston et al (1975) described 8 patients who had developed a severe

stricture from reflux oesophagitis. The oesophagitis appeared relieved by an antrectomy and Roux en Y procedure (Follow-up 11-12 months). Definite improvement was seen with regard to complaints of dysphagia and substernal pain. This operation was therefore strongly advised for patients for whom an earlier antireflux operation had failed.

**4. Relationship between macroscopic and histological findings in reflux oesophagitis.**

During endoscopic examination, normal mucosa is often seen in the oesophagus of patients with gastro-oesophageal reflux. Initially it was thought that these patients did not have an oesophagitis.
Svoboda et al (1967) and Ismail-Beigi et al (1970), pointed out that a normal endoscopic appearance and the hostology of biopsy specimens taken from this socalled "normal" mucosa were not always in agreement. The latter performed a study in which four groups of patients (i.e. patients with or without clinical symptoms of reflux and patients with or without objectively demonstrable oesophagitis) were compared. Endoscopy and biopsy were performed on each patient. The oesophageal biopsy was regarded as normal if the thickness of the basal cell-layer was less than 15% of the total epithelial thickness, and no inflammatory exudate was present in the lamina propria. Ismail-Beigi et al (1970) studies 43 patients with definite clinical symptoms of oesophagitis and 21 patients without symptoms. On endoscopy, 34 showed macroscopic signs of oesophagitis, and in 9 no abnormality was seen. However, in 7 of these 9 patients definite histological changes were present, in particular hyperplasia of the basal cells, increase in papillary height and acute inflammatory exudate in the lamina propria. Of the patients ($n = 21$) who showed neither clinical nor objective characteristics of reflux, two showed an abnormal histological picture.
Kobayashi and Kasuqui (1974) studied 144 patients in which the endoscopic and histological findings were compared. Only patients with definite clinical symptoms of reflux were examined. Oesophageal mucosa hyperaemic on endoscopy was regarded as mild oesophagitis, erosion without exudate qualified as moderate, and ulceration and white exudate surrounded by red mucosa as severe oesophagitis. Mucosal granulation with reduced elasticity of the oesophageal wall suggested chronic or late oesophagitis. Leukoplakia was regarded as an oesophagitis following hyperplastic changes of the mucosa. 48 of 144 patients showed a normal oesophageal mucosa on endoscopy, 16 (33%) of these patients had indeed an abnormal histological appearance, particularly when papillary height and hyperplasia of the basal

cell layer were taken into account. Both of which were often found in the presence of leucocytic infiltration of the lamina propria. Of the remaining 96 patients with endoscopically diagnosed oesophagitis, histologically oesophagitis was found in 90%. The 10% of patients in which oesophagitis was diagnosed endoscopically, but who had no histological abnormality, was that group of patients which showed a hyperaemic mucosa. This suggests that an oesophageal mucous membrane, which looks hyperaemic on endoscopy (staged mild oesophagitis), does not always show histological abnormalities. On the other hand endoscopically normal mucosa can show in an appreciable number of cases definite histological changes.
Behar and Sheahan (1975) studied multiple biopsies of 15 asymptomatic patients, and 40 patients with definite symptoms of reflux. 8 (20%) of the 40 patients with clinical symptoms of reflux had an oesophageal mucous membrane of normal appearance on endoscopy. 38 (95%) of the symptomatic patients showed at least two abnormal oesophageal biopsies. (Increase in papillary height, hyperplasia of the basal cell layer, and acute inflammatory exudate in the lamina propria). Only 1 of the 15 asymptomatic patients showed an abnormal histological picture.

Summarizing, it can be concluded that the endoscopical diagnosis of oesophagitis, especially the mild form, is difficult and should always be supplemented with biopsy examination for correct diagnosis.

## B. PERSONAL STUDY

CHAPTER III

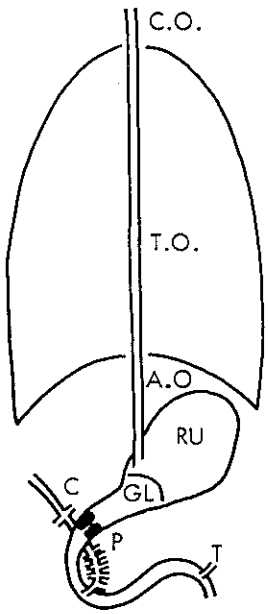
# METHODS AND MATERIALS

### 1. Laboratory animals.

Male WISTAR rats, 10-12 weeks old with a weight of 240-300 gram were used for the experiments.

### 2. Anatomy of the rat oesophagus (fig. 1.).

The rat oesophagus is about 8 cm long and can be divided into a cervical, a thoracic and an abdominal portion. Compared to man the abdominal




C.O. = Cervical oesophagus
T.O. = Thoracic oesophagus
A.O. = Abdominal oesophagus
C = Common bile duct with distal the pancreatic ducts
RU = Rumen of the stomach
GL = Glandular portion of the stomach
T = Lig. of Treitz
P = Pylorus

Fig. 1. Schematic representation of the oesophagus, stomach, duodenum, common bile duct and pancreatic ducts.

segment of the oesophagus in the rat is relatively long (1-1.5 cm). In contrast to the human oesophagus, the oesophagus of the rat consists of squamous epithelium and has a striated muscule layer (see Chapter VI.1). The epithelium of the human oesophagus has no horny layer and only the proximal part of the oesophagus has a striated muscle layer. The rat stomach consists of the glandular stomach and the rumen (the so called pre-stomach). The cardia of the stomach is found in the middle of the lesser curve, on the transition zone between the rumen and the glandular portion. The thin walled rumen has the same mucosal covering as the oesophagus. The glandular stomach is thick walled and has a mucous membrane consisting of cubical cells and simple tubular glands.

### 3. Experimental groups.

In order to achieve gastric reflux (G), reflux of pancreatic juice (P), bile (B), and combinations of these in the oesophagus, different experimental groups were formed. In total 9 experimentel groups were tested. Seven groups were formed such that after surgical reconstruction reflux of gastric, pancreatic and bile separately or in combination could occur. These groups were compared with a group of rats with oesophago-jejunostomy and a jejuno-jejunostomy according to Roux en Y and a sham operated group. After a pilot study of rats devided randomly, the required number of rats to be operated upon for the nine experimental groups was defined (see chapter IV. 2). These rats again were divided randomly. A total of 195 rats were included in these experiments.

### 4. Operative techniques.

*General.*

Inhalation anesthesia of ether and air was used in all experiments. The abdominal wall of all rats was shaved before a median laparotomy from the xiphoid process to the level of the umbilicus was performed. Care was taken not to open the pleural cavity. Anastomosis of oesophagus and intestines and stomach was carried out in one layer with continuous 7.0 silk stitches in all cases. The cardia was ligated with 4.0 silk. The abdominal cavity was closed with continuous 4.0 catgut. The skin was closed with metal clips. The specific surgical techniques used for each of the various groups were as follows:

a. *Sham group (n* = 10): In this group a laparotomy alone was carried out.
b. *G* + P + B + group (n = 21): oesophago-jejunostomy, stomach in situ (see fig. 2).

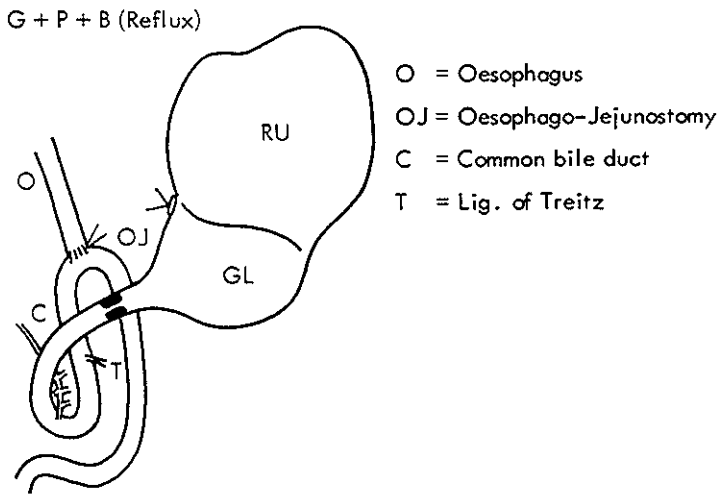



Fig. 2. G + P + B group (oesophago-jejunostomy, stomach in situ).

After laparotomy, the liver lobes were retracted and the parietal peritoneum of the anterior and lateral sides of the oesophagus was opened, the left gastric artery and the vagal nerves were then dissected from the lateral side of the oesophagus, over a distance of about 10 mm. After division of the oesophagus just above the cardia, the cardia was ligated with 4.0 silk. On the lateral sides of the oesophagus stay sutures were placed, and a small loop of the jejunum just beyond the ligament of Treitz was mobilized. An opening 5 mm wide was made in this loop at a distance ± 4 cm beyond the ligament of Treitz. An end-to-side oesophago-jejunostomy was then carried out in one layer using continuous stitches. For this purpose the lateral sides of the oesophagus and the jejunum were fixed, first the anterior side of the anastomosis was carried out and then the posterior side. In this way, reflux consisting of a mixture of bile, pancreatic and gastric juice could occur.

c. *B + P group (n = 22): Total gastric resection and oesophago-jejunostomy (see fig. 3).*

The operative procedure here was identical to that of group G + P + B, however, a total gastric resection including the pylorus was carried out in addition (see fig. 4). In order to achieve this, the circulation to the stomach was first clamped. The left gastric artery was tied off ± 10 mm above the cardia. Next, the right gastro-epiploic vessels (with the exception of branches to the omentum and pancreas) were ligated. Care was taken in the region of pylorus not to damage the gastro-duodenal artery. The gastric vessels and the

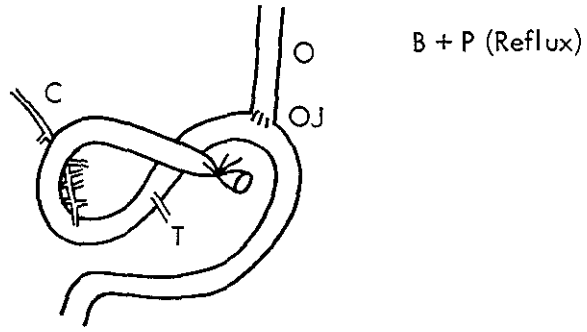



Fig. 3. B + P group (total gastric resection and oesophago-jejunostomy).

pyloric artery were electro-coagulated. Next the stomach was removed. During the procedure no care was taken to preserve the vagal nerves. After total gastric resection, the duodenum just beyond the pylorus was closed with a silk ligature and an oesophago-jejunostomy was carried out. After this procedure only reflux of bile and pancreatic juice could occur.

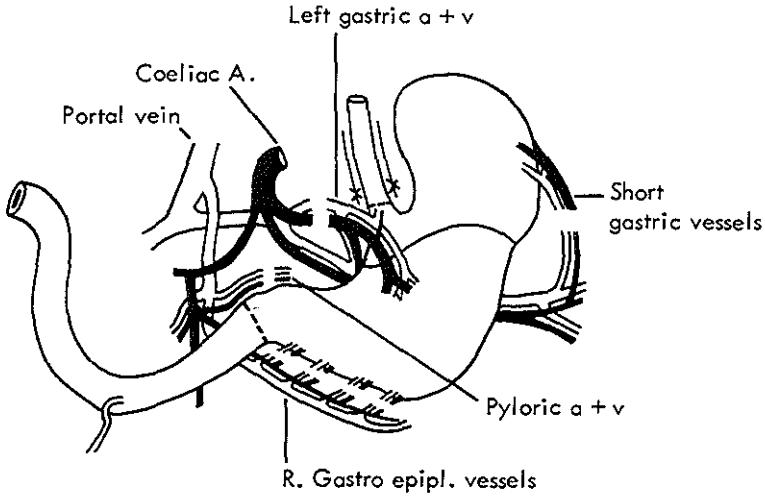



Fig. 4. Division of large vessels of the stomach (diagrammatic) and the resection planes carried out (interrupted lines).

d. *P + G group (n = 24): Oesophago-jejunostomy and biliary diversion, with preservation of the stomach (see fig. 5).*

Here again an oesophago-jejunostomy was performed and in addition a common bile duct diversion. Fig. 6 (A-D) shows the operative technique used to achieve the common bile duct diversion. Prior to the diversion procedure, the common bile duct was double ligated just proximally to the main pancreatic ducts (A). After approximately 60 min. of total obstruction, the common bile duct procimal to the ligature became dilated, thus rendering achievement of the "anastomosis" easier. The common bile duct was then divided (B). A loop of jejunum 25 cm distally to the oesophago-jejunostomy was brought up to the liver hilus and a 1 mm wide stab wound made (C). Next the common bile duct was pulled into the jejunum with the help of 7.0 silk suture and then fixed (D).

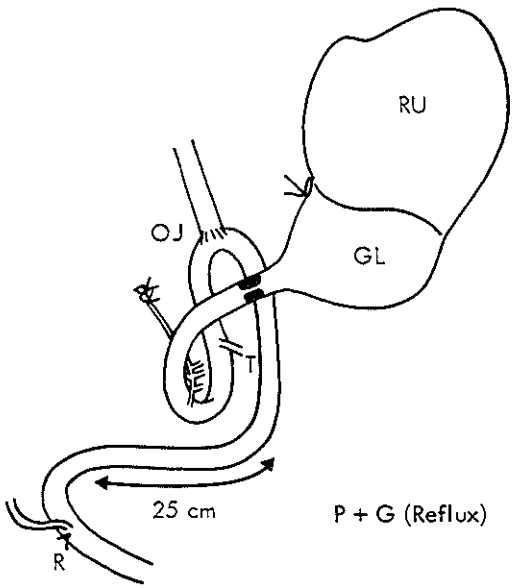



Fig. 5. P + G group (oesophago-jejunostomy and biliary diversion with preservation of the stomach).

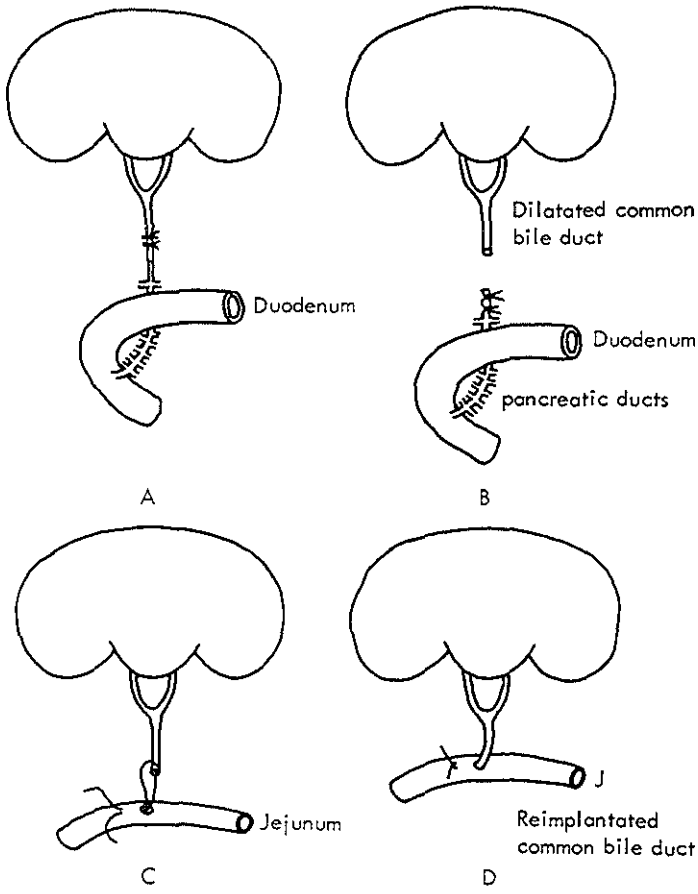



Fig. 6. Deviation method of common bile duct.
A. ligated common bile duct,
B. divided dilatated common bile duct,
C. stabwound jejunum, with the silk suture in the common bile duct,
D. common bile duct pulled into the jejunum.

e. *P group (n = 22): Total gastric resection with oesophago-jejunostomy and biliary diversion (see fig. 7).*

In this group a total gastric resection and an oesophago-jejunostomy was carried out and next the common bile duct was "anastomosed" to the jejunum, 25 cm. distally from the oesophago-jejunostomy.

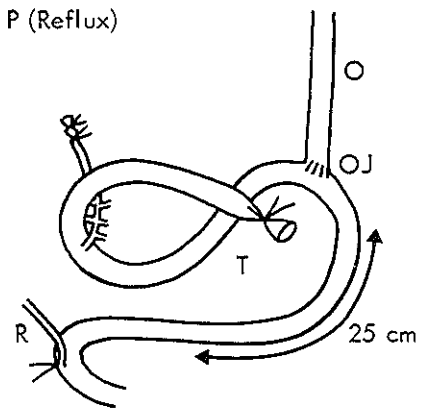



Fig. 7. P group (total gastric resection with oesophago-jejunostomy and biliary diversion).

f. *G + B group (n = 19): Oesophago-jejunostomy with choledocho-jejunostomy and gastro-jejunostomy (see fig. 8).*

In this group the ventral side of the glandular part of the stomach and the common bile duct were anastomosed to the same loop of the proximal jejunum and a distal jejuno-jejunostomy was performed. The distance between the oesophago-jejunostomy and jejuno-jejunostomy amounted to 25 cm.

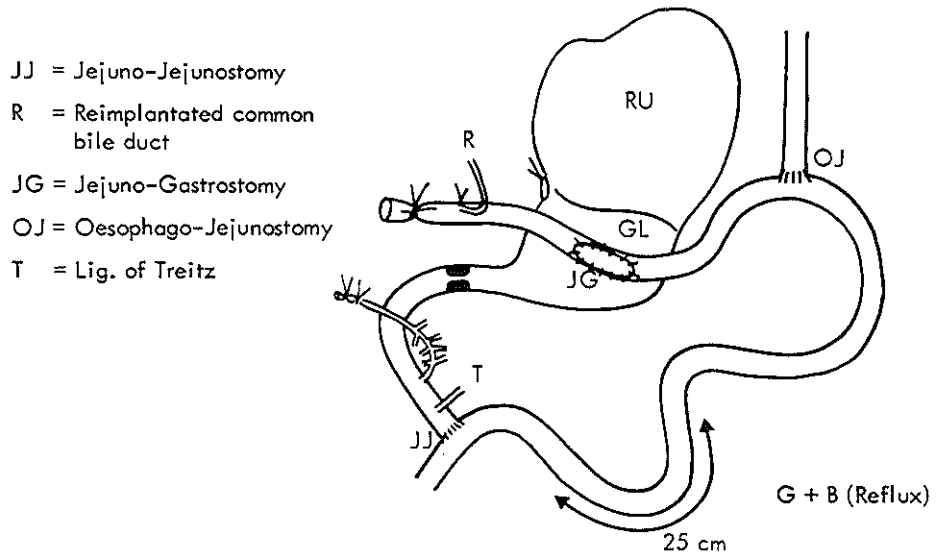



Fig. 8. G + B group (oesophago-jejunostomy with choledocho-jejunostomy, gastro-jejunostomy and jejuno-jejunostomy).

g. *G group (n = 19): Oesophago-jejunostomy, gastro-jejunostomy and jejuno-jejunostomy (see fig. 9).*

In this group the ventral side of the glandular part of the stomach was anastomosed to a loop of proximal jejunum. Distal to this an oesophago-jejunostomy and finally, a jejuno-jejunostomy was performed.

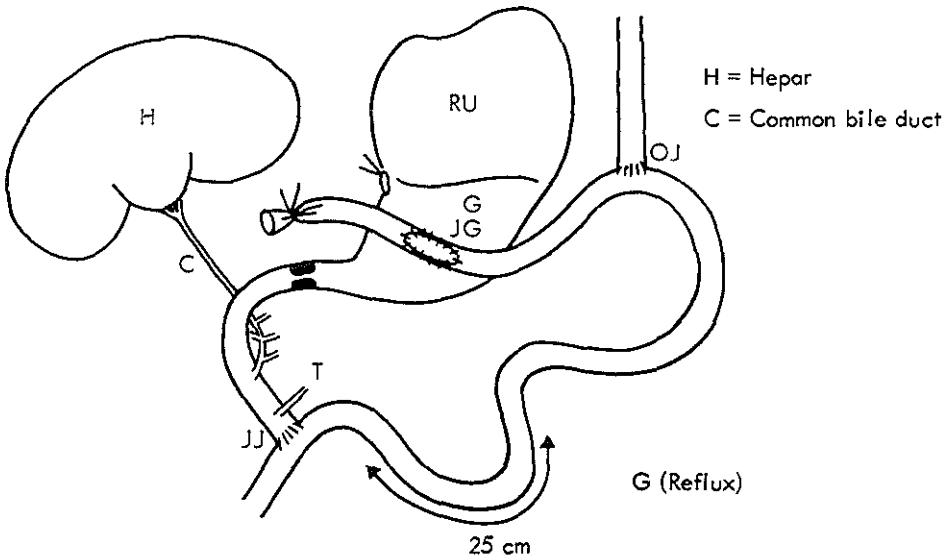



Fig. 9. G group (oesophago-jejunostomy, gastro-jejunostomy and jejuno-jejunostomy).

h. *B group (n = 19): Oesophago-jejunostomy with choledocho-jejunostomy and jejuno-jejunostomy (see fig. 10).*

In this group the common bile duct was anastomosed to a proximal jejunal loop and then an oesophago-jejunostomy and a jejuno-jejunostomy were carried out.

i. *Roux en Y-group (n = 19): Oesophago-jejunostomy and jejuno-jejunostomy (see fig. 11).*

In this group a classical oesophago-jejunostomy and a jejuno-jejunostomy (end-to-side) were carried out. The second anastomosis was performed 25 cm distally to the first anastomosis.

In group G and G + B, the pylorus was left intact and theoretically reflux from the duodenum into the stomach was possible. Exclusion of this potential error could not be prevented and will be discussed in chapter VII.

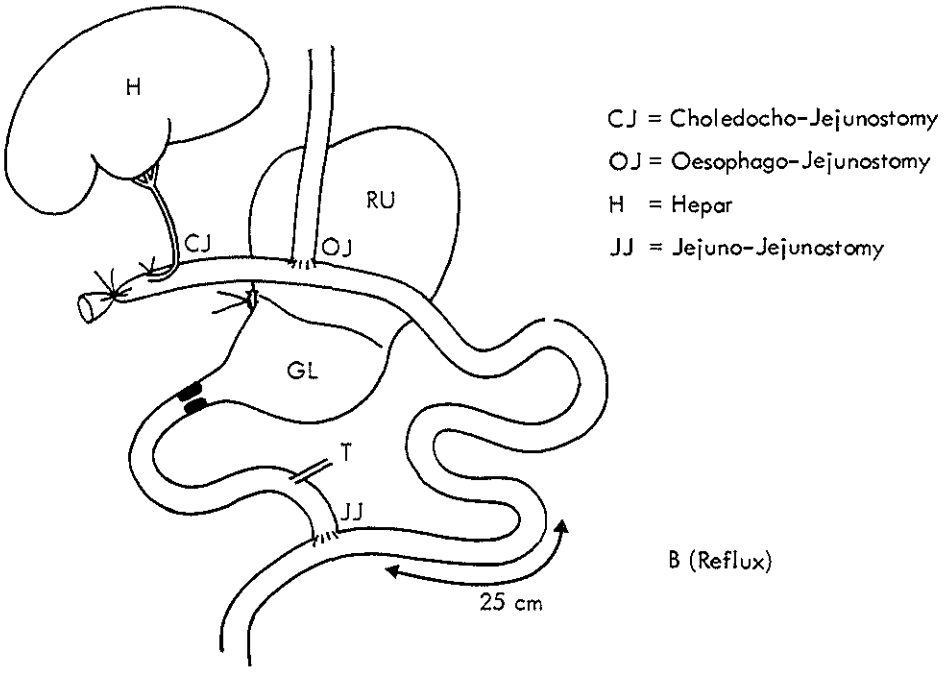



Fig. 10. B group (oesophago-jejunostomy with choledocho-jejunostomy and jejuno-jejunostomy).

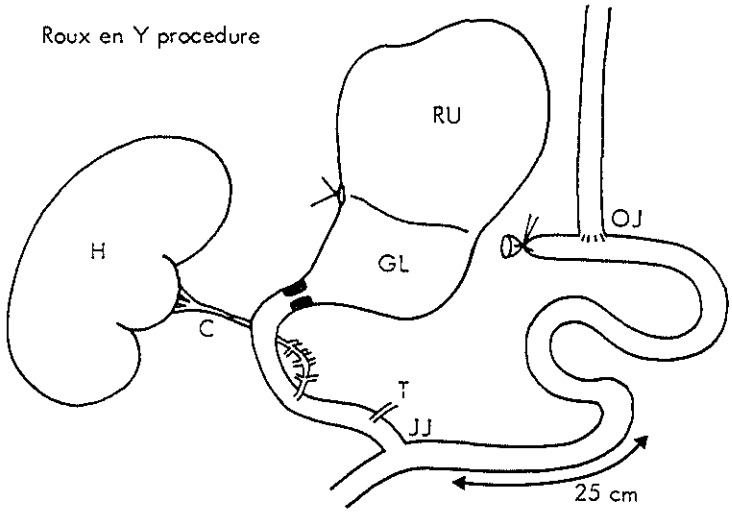



Fig. 11. Roux en Y group (oesophago-jejunostomy and jejuno-jejunostomy).

**5. Post-operative care.**

Post-operatively the rats were placed under an infrared lamp for about one hour, by which time they had recovered from anaesthesia. They were then placed in two's, in sterilised cages at room temperature and constant humidity. A standard diet (Hope Farms B.V., Woerden) without restriction was administered throughout the experiment. Water with a pH of 3.3 was given ad libitum. The rats received no antibiotic treatment. Weighing and evaluation of the post-operative course were carried out twice weekly. Activity of the rats, development of icterus, abdominal distention and dyspnoe were all registered. On day 3, 5, 7, 14, 28 and 42 after operation a small number of rats from every experimental group was sacrificed (see table 2, chapter IV.2).

**6. Autopsy.**

From all experimental groups animals were killed sequentially on day 3, 5, 7, 14, 28, 42. Immediately after, autopsy was performed. The abdominal cavity was opened and inspected and the organs were individually examined. In the presence of fluid, the colour and aspect (clear or cloudy) were noted. If fluid was present, the source was sought (leakage or peritonitis). The volume of fluid was not measured. The anastomoses were inspected, and special attention was paid to the presence of leakage or stenosis. Furthermore, the jejunum distal to the oesophago-jejunostomy was checked for dilatation. Dilatation of the proximal jejunum loop or common bile duct (if deviated) was estimated by means of callipers, and compared with the diameter of a normal proximal jejunum loop or common bile duct respectively (the normal diameter of a proximal jejunum loop is 3-4 mm, normal diameter of the common bile duct 0.6-1.0 mm). After inspecting the abdominal cavity, a thoracotomy was performed, and the thoracic part of the oesophagus was examined. Oesophageal perforation(s) and mediastinitis being sought for. Thereafter the whole oesophagus including the oesophago-jejunostomy was excised (see Fig. 12). The stretched length and breadth were measured and noted. If the oesophagus was less elastic than normal, this was noted (the normal rat oesophagus can be stretched approximately 1½ cm longitudinally). The degree of loss of elasticity was not determined. Next a silastic canule with a diameter of 4 mm (the maximum diameter which can be passed through a normal rat oesophagus) was introduced into the oesophagus, to ascertain whether or not stenosis was present. This was noted on a standard form (see fig. 13). The degree of stenosis was not assessed. The oesophagus was then opened longitudinally on the median ventral side,

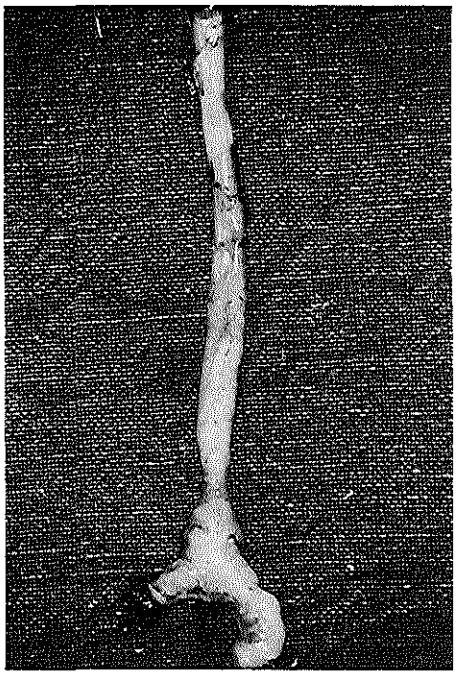


Fig. 12. Excised oesophagus, including oesophago-jejunostomy.

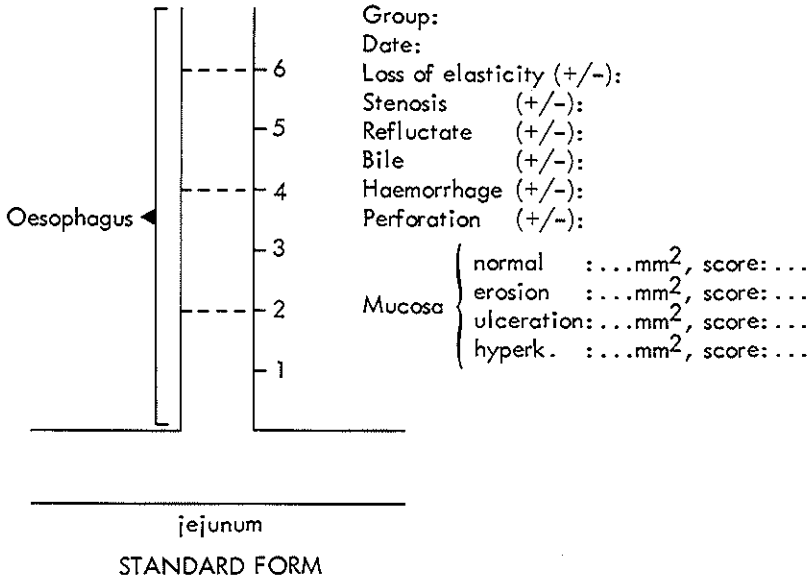



Fig. 13. Schematic representation of the oesophagus and the oesophago-jejunostomy, opened longitudinally on the median ventral side and folded outwards. The oesophagus is divided schematically into three equal parts.

folded outwards, stretched, and pinned onto a cork block. The distal 6 cm of the oesophagus was examined. Food retention and the presence of bile pigment were noted. Perforation(s) and bleeding of the mucous membrane were sought for. Erosions and/or ulcerations and/or white hyperkeratosis (for definition see chapter IV) were then measured and noted with particular care.

**7. Scoring system of oesophageal lesions.**

As already described, the lesions of the oesophageal mucosa i.e. erosion, ulceration or hyperkeratosis were measured by means of callipers and magnifying glass. Since the lesions were relatively superficial and demonstrated no tendency to perforation, the degree of the oesophagitis could best be quantified by measuring the surface area of the lesions. The distal 6 cm of the oesophagus were examined (the total length varied between 7-8 cm). The lesions, usually irregular in form were drawn in on the standard form (fig. 13) and then enclosed in a triangle or rectangle, depending on the shape of the lesion. The surface area of the triangle or rectangle was determined. The relationship of the surface area of the triangle or rectangle to the total surface area of the oesophagus was calculated. The surface area of the lesions were scored from 0-15. 15 being the surface of the total oesophagus. An oesophagus with 30% ulcerated, and 70% normal mucous membrane was scored: 0.3 x 15 = 4.5 ulceration and 0.7 x 15 = 10.5 normal. To get an idea which part of the oesophagus was most seriously damaged, the oesophagus was divided into three equal parts, the proximal, middle and distal part. The distal part including the oesophago-jejunostomy. The maximal score for each part was 5. To illustrate this method of scoring, an example is given in which the lesions are enclosed in rectangles (see fig. 14). The score for the whole oesophagus (maximum score 15) is 80 : 720 x 15 = 1.67 ulcerative, 72 : 720 x 15 = 1.50 erosive, and 15 - 1.67-1.50 = 11.83 normal. When the lesions of the proximal, middle and distal part of the oesophagus are scored individually; the distal part is 1.67 ulcerative, 3,33 normal and 0 erosive; middle part is 1.50 erosive, 3.5 normal and 0 ulcerative; proximal part is 5 normal, 0 erosive and 0 ulcerative.

To be able to compare the lesions found in the experimental groups, the individual scores of the different lesions were either separately or together added, and a sumscore and a mean sumscore were calculated. Thus by adding the individual erosion score and ulceration score, an erosion + ulceration sumscore could be obtained. Then a mean sumscore of erosion + ulceration for every experimental group was calculated. In the example quoted the

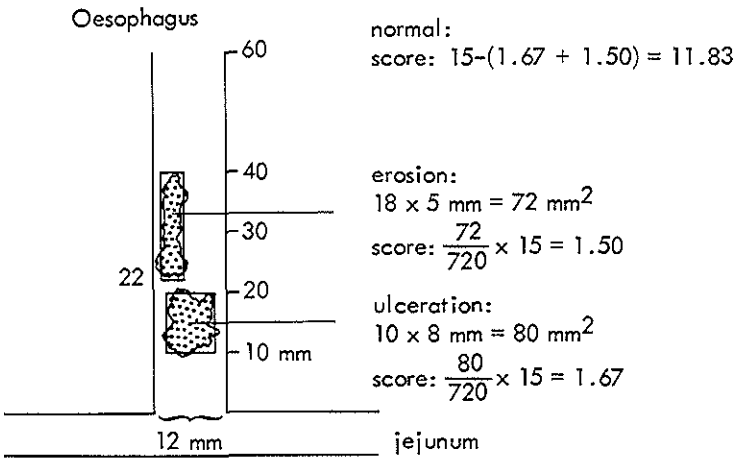



Fig. 14. An example of an erosion and an ulcer in an oesophagus and the manner of determination of the scores for the whole oesophagus (erosion score 1.50, ulceration score 1.67, erosion + ulceration sum score 3.17).

erosion + ulceration sumscore is 1.67 + 1.50 = 3.17 for the whole oesophagus.

**8. Histological techniques.**

After macroscopic examination of the oesophagus and charting the lesions, two or more strips of oesophagus were cut from representative places for histological study. These pieces were then pinned to a cork block. The place where a cut for histological inspection had to be made, was marked in ink on the cork block. The strips were then fixed in phosphate buffered 2% formaldehyde solution (pH: 7.0). Next the fixed tissue was dehydrated in progressively stronger alcohol solution (70-100%) and then in xylol. The fixed and dehydrated oesophageal specimens were thus imbedded in a paraffin-paraplast beeswaxmixture at 57° C, so that full length cuts could be made. Then from all pieces at least two full length slices were cut (thickness 3-4 $\mu$). These slices were stained with hematoxilin-azophloxin-saffran (HAS) and a masson trichroom-dye.

**9. Radiographic examination.**

Seven days post-operatively two rats were taken from each group to ascertain whether or not there was radiological evidence of reflux. Under light ether-air anaesthesia a silastic cannula (2 mm cross-section) was brought into the stomach via a small midline laparotomy.

If a total gastric resection had been carried out, the cannula was placed in the duodenum. In the B group and the B + G group, the silastic cannula was placed in the proximal jejunum loop pointing in the direction of the oesophago-jejunostomy, just proximal to the bile duct. About 2 ml of a mixture of micropaque and gastrograffin (1 : 1) was injected under constant manual pressure through the cannula. Radiological pictures were made after injection of the contrast. The passage of the contrast fluid was followed by floroscopy. As control 5 normal rats were tested. A cannula was brought into the stomach via the oesophagus, and 2 ml contrast medium injected. This group served to verify the absence of gastro-oesophageal reflux in normal rats. After the stomach of these rats was filled with contrast fluid, the cannula was removed from the oesophagus. In these rats reflux was tested by placing the rats in Trendelenburg position and raising the abdominal pressure by manual compression of the abdomen.

CHAPTER IV

# GENERAL RESULTS OF THE STUDY

## 1. Duration of operations.

The operating time per rat was, dependant on the number of anastomoses performed. The choledocho-jejunostomy requiring the most. For the G + P + B group, where only an oesophago-jejunostomy was performed, the operation time amounted to an average of 20 min. For the B + G group where an oesophago-jejunostomy, choledocho-jejunostomy, gastro-jejunostomy and a jejuno-jejunostomy were carried out, an average of 70 min operating time was necessary. The average duration of operation for the other groups lay between these two limits.

## 2. Experimental groups.

In total 195 rats were operated upon. The different groups and the number of animals which died from complications during the experiments are given in table I.

Table 1. Number of rats of the different experimental groups operated upon, including the number of deaths and the day of death.

| Group | Number operated upon | Number of deaths | Day of death |
|---|---|---|---|
| G + P + B | 23 | 2 | 7, 14 |
| P + B | 24 | 2 | 1, 4 |
| P + G | 27 | 3 | 1, 4, 13 |
| P | 24 | 2 | 4, 5 |
| G + B | 22 | 3 | 7, 10, 14 |
| G | 21 | 2 | 3, 11 |
| B | 21 | 2 | 7, 9 |
| Roux en Y | 23 | 4 | 14, 15, 21, 28 |
| Sham | 10 | — | — |
| Total | 195 | 20 | |

Except for the SHAM group, all early deaths occured as a result of complications. The mortality amounted to 10,2%, and was evenly distributed over the various experimental groups. As described in Chapter III the remaining 175 rats were sacrificed sequentially on day 3, 5, 7, 14, 28 and 42. The number of rats sacrified are given in table 2, arranged according to the time post-operatively. The rats dying from complications are not mentioned in this table.

Table. 2. Number of rats of the different experimental groups, sacrificed sequentially on day 3, 5, 7, 14, 24 and 42.

| | DAYS post-operatively | | | | | | |
|---|---|---|---|---|---|---|---|
| Group | 3 | 5 | 7 | 14 | 28 | 42 | Total |
| G + P + B | 5* | 4 | 4 | 2 | 4 | 2 | 21 |
| P + B | 3 | 3 | 5 | 4 | 4 | 3 | 22 |
| P + G | 5 | 2 | 5 | 4 | 4 | 4 | 24 |
| P | 5 | 3 | 4 | 4 | 3 | 3 | 22 |
| G + B | 2 | 2 | 4 | 3 | 4 | 4 | 19 |
| G | 2 | 2 | 4 | 3 | 4 | 4 | 19 |
| B | 2 | 2 | 4 | 3 | 4 | 4 | 19 |
| Roux en Y | 2 | 2 | 4 | 3 | 4 | 4 | 19 |
| SHAM | — | — | — | — | — | 10 | 10 |
| Total | 26 | 20 | 34 | 26 | 31 | 38 | 175 |

* Number of rats sacrificed.

### 3. Body weight after operation.

The rats were weighed twice a week, from the moment just before the operation to the time that they were sacrificed. The average body weight of the still living animals of each experimental group was calculated on day 0, 7, 14, 28 and 42 (see table 2), and expressed as a percentage of the average pre-operative body weight. All lost weight in the immediate post-operative period (see fig. 15). The group of rats in the laparotomy group, reached their initial weight within two weeks. From then on they gained in weight. Rats from the B + G, G, B, Roux en Y groups reached their preoperative weight

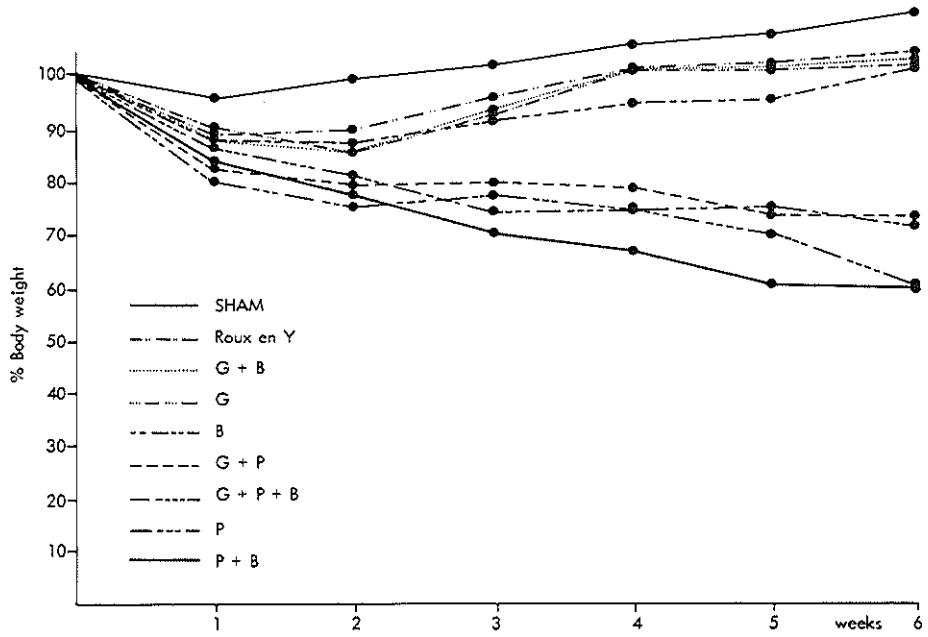



Fig. 15. Body weight as a percentage of initial weight of the still living animals, with respect to time in weeks.

after 4-6 weeks. These were the groups where on autopsy no oesophagitis was found.

The remaining groups continued to lose weight, losing about 40% of initial weight by the end of six weeks. Until the third post-operative week all animals showed similar weight loss. After this two week period the weight curves reflected the presence of oesophagitis quite acurately.

### 4. Serum Bilirubin.

To define the normal serum bilirubin value in rats, total bilirubin was estimated in 50 normal rats. After statistical analysis the normal total bilirubin value was found to be below 3.2 $\mu$ mol/l. A total bilirubin value greater than 3.2 $\mu$ mol/l was thus regarded as raised..

The common bile duct diversion was performed in 94 rats (see chapter III. 4) from group P + G, P, B + G and B. Bilirubin values were estimated on the day of operation and on the day of autopsy. Early in the experiment, only two rats developed progressive jaundice, one rat out of group B and one out of group B + G. This lead to their deaths on the 9th and 10th post-operative day (see chapter IV. 6). Post-operative jaundice was observed by the second post-operative day in them. Another two rats one out of group B and one out of

group B + G, also showed a raised serum bilirubin at the time of death, dehiscence of the reimplanted common bile duct being found at autopsy. These two rats died on the 7th post-operative day as a result of biliary peritonitis (see chapter IV. 6). In the remaining 90 rats the bilirubin remained normal. Of these, three died spontaneously of other causes. These rats were definitely not jaundiced post-operatively and at autopsy appeared to have an unhampered flow of bile.

In summary in 94 rats the bile duct was reimplanted, optimal biliary flow was present in 90 of them. The diversion technique used in this experiment proved to be quite acceptable, with few technical failures.

**5. Radiographic examination.**

All rats examined (two from each experimental group), except the Roux en Y and SHAM group, showed definite reflux of contrast medium through the oesophago-jejunostomy into the oesophagus. Moreover the radiographic pictures showed dilation of the oesophagus above the hiatus in all the rats from the G + P + B, P + B, G + P, and P group. This dilated portion of the oesophagus correlated with the region of most extensive oesophageal lesions on autopsy. The five normal control rats showed no reflux of contrast medium, when the rats were tilted to Trendelburg position or when the intra-abdominal pressure was raised by manual compression on the abdominal wall.

**6. Complications.**

20 of the 195 rats suffered from complications, which led to their early deaths. The number of rats and cause of death are described in table 3.

Table 3. Causes of death and number of rats which died.

| | | |
|---|---|---|
| Post-operative bleeding | | : 2 |
| Aspiration pneumonia | | : 7 |
| Diffuse peritonitis following } | perforated oes-jejunostomy | : 6 |
| | dehiscence of common bile duct anastomosis | : 2 |
| Progressive jaundice as a result of common bile duct obstruction | | : 2 |
| Unknown | | : 1 |
| | Total | 20 |

Table 4 gives the distribution of causes of death over the different experimental groups, together with the days post-operatively the animals died.

Two rats died the first post-operative day. Autopsy showing intra-abdominal haemorrhage as the cause of death. The seven rats which developed pneumonia suffered a progressive shortness of breath and reducted activity for some days before dying. The rats that developed peritonitis, became progressively indolent with increasing abdominal swelling. Death due to biliary peritonitis occured some days later, than death due to perforation of the oesophago-jejunostomy. Two rats developed jaundice in the immediate post-operative period (see chapter IV. 4).

Table 4. Distribution of causes of death over the different experimental groups, together with the days post-operatively the animals died.

| Group. | No. of Death. | Cause of death and time after operation. |
|---|---|---|
| G + P + B | 2 | Diff. perit as result of perforated oes-jej (day 7), asp. pneumonia (day 14). |
| B + P | 2 | Post. op. bleeding (day 1), diff. perit as result of perf oes-jej (day 4). |
| P + G | 3 | Post. op. bleeding (day 1), diff. perit as result of perf oes-jej (day 4), asp. pneumonia (day 13). |
| P | 2 | Diff. perit. as result of perf. oes. jej. (day 4 and 5). |
| B + G | 3 | Diff. perit as result of dehiscence of common bile duct (day 7), progr. jaundice (day 10), asp. pneumonia (day 14). |
| G | 2 | Diff. perit as result of perf. oes-jejunostomy (day 3), asp. pneumonia (day 11). |
| B | 2 | Diff. perit. as result of dehiscence common bile duct (day 7), progressive jaundice (day 9). |
| Roux en Y | 4 | Asp. pneumonia (day 14, 15, 21), unknown (day 28). |
| SHAM | 0 | ——— |

## 7. Autopsy findings.

### *7.1. Abdomen.*

*Post-operative bleeding*: two rats died on the first post-operative day from intra-abdominal haemorrhage, which caused death. Related to inadequate haemostasis during operation. These rats were excluded from further evaluation of oesophageal lesions.
*Peritonitis*: 8 rats which died early, due to peritonitis (see chapter IV. 6). This was due to perforation of the oesophago-jejunostomy in 6 cases, probably due to a technical fault. An abcess and food remains were found near the oesophago-jejunostomy. The abdominal cavity contained cloudy fluid. In two further cases diffuse peritonitis appeared to be the result of dehiscence of the choledocho-jejunostomy. Here green bilious fluid was found throughout the abdominal cavity. No signs of peritonitis was found in any of the remaining rats.
*Intestinal anastomoses*: at autopsy the intestinal anastomoses (jejuno-jejunostomu and gastro-jejunostomy) appeared functional, and without sign of leakage or stenoses in all. The jejunal loop distal to the oesophago-jejunostomy showed dilatation in all cases. The diameter was always 2-3 times greater than that of the proximal jejunum.
*Bile duct*: as already mentioned, biliary diversion was performed in 94 rats. Two rats jaundiced and died in just over a week. At autopsy in each a grossly dilated common bile duct was found. The liver too was enlarged and green ascites fluid was present in the abdomen. Dehiscence of the bile duct from the jejunum, was the direct cause of death in a further two cases. Good biliary flow was found in 90 rats (95,7%), as born out by normal serum bilirubin levels. In these remaining cases, however, the common bile duct was dilated (1,2-5 mm) normally 0,6-1 mm. (see fig. 16 and fig. 17). Whether this dilatation was due to a partial obstruction at the bilio-jejunal "anastomoses" or not, is not clear.

### *7.2. Oesophagus*

After laparotomy, thoracotomy was carried out and the thoracic part of the oesophagus was inspected. In no case was oesophageal perforation or mediastinitis found. The whole oesophagus together with the oesophago-jejunostomy was excised.
*Loss of elasticity*: the oesophagus of the rats in the B + P + G group, the B + P group, the P + G group and the P group appeared white on the outer surface, especially distally. This decolorization was even more marked after

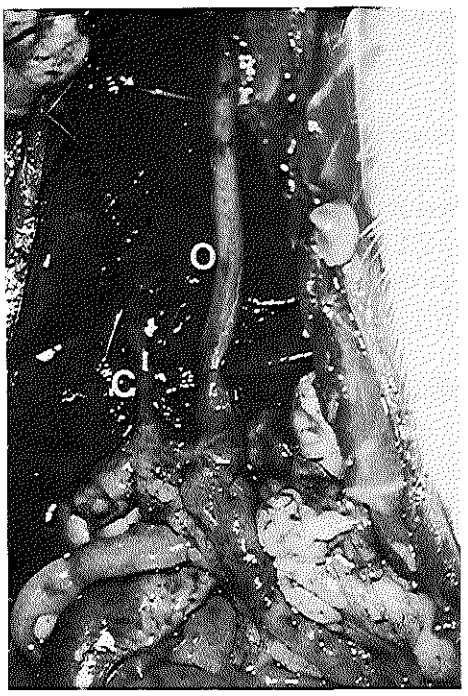



Fig. 16. Autopsy findings in a rat from group B (bile reflux), in which an oesophagojejunostomy, a choledocho-jejunostomy and a jejuno-jejunostomy was performed. c = common bile duct, o = oesophagus, which are anastomosed to the same jejunum loop. The common bile duct is dilated.

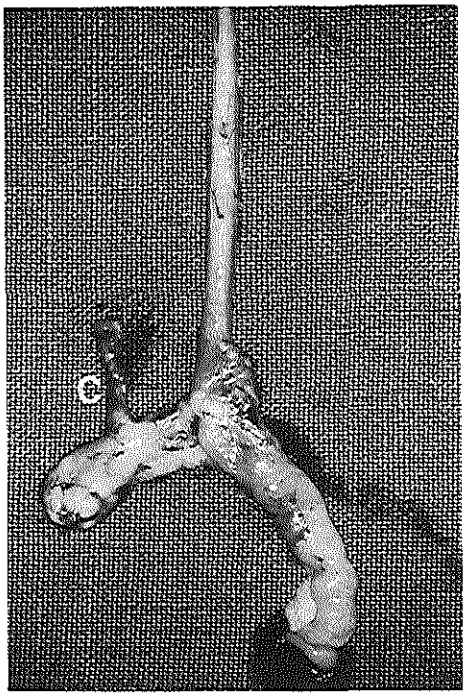


Fig. 17. Excised proximal jejunum loop, including common bile duct and oesophagus of the same animal as given in the figure 16. c = common bile duct.

42 days. After 28 days every rat in these groups showed definite loss in elasticity of the oesophageal wall, in both longitudinal and lateral directions. This loss of elasticity appeared further increased after 42 days. There was no essential difference in loss of elasticity between any of the above mentioned groups. The oesophagus of the rats in the other groups did not show any changes.

*Shortening*: the oesophagi from the rats of the B + P + G group, the B + P group, P + G group and P group showed a progressive reduction in length as well in width. After 28 days an average shortening of 0.5-0.75 cm was present. After 42 days this shortening was more marked (0.75-1 cm). Again no

shortening of the oesophagus was found in any of the rats in the other experimental groups.

*Aspiration pneumonia and stenosis*: 7 rats died early in the experiment due to aspiration pneumonia. In 5 rats which suffered early death due to aspiration pneumonia, passage of a silastic canula (cross section 4 mm) through the oesophago-jejunostomy was not possible, i.e. a true stenosis. Aspiration pneumonia was clear from the appearance of the lungs of these animals. This stenosis was found in the G + P + B (n = 1), P + G (n = 1), B + G (n = 1), B (n = 1) and Roux en Y (n = 1) group..
In 3 out of the 5 rats the stenosis was caused by fault in technique, in two cases by granuloma formation at the side of the anastomosis. Stenosis occurred whether oesophagitis was present (rats from group G + P + B and P + G) or not (rats from group B + G, B and Roux en Y). In all cases there was marked dilatation of the oesophagus proximal to the stenosis. In two other rats definite signs of pneumonia were found at autopsy, without evidence of oesophageal stenosis, oesophageal dysfunction probably played a role in these cases. In all the remaining rats no stenosis in the oesophagus was found.

The oesophagus was then opened anteriorly in the midline throughout its length and folded outwards.
In the 7 rats which died early in the experiment due to aspiration pneumonia (see chapter IV. 6), remains of food particles and hair were found in the oesophagus. In rats in group B and B + G, dark green bile staining was to be seen on the oesophageal mucous membrane. Never intramucosal haemorrhages were found.

*Oesophageal mucosal lesions*: The oesophagus was opened anteriorly in the midline throughout its length and folded outwards. Many mucosal lesions were apparent in the P, P + B, G + P + B and the G + P groups. No lesions were found in the other experimental groups. The lesions were carefully estimated with the aid of magnifying glass and callipers and drawn in on the standard form (see chapter III, fig. 13). Besides normal oesophageal mucosa, erosions, ulcerations and hyperkeratotic changes were found. A fully intact mucosa that looked glistening greyish white, was called normal (see fig. 18). Dull grey granular mucosa with superficial defects was regarded as an erosion. In the case of ulceration, the mucosa was absent and a granular red wound base was seen (see fig. 19). Hyperkeratotic changes were defined as white patches with elevated areas of increased keratin formation (see fig. 20).

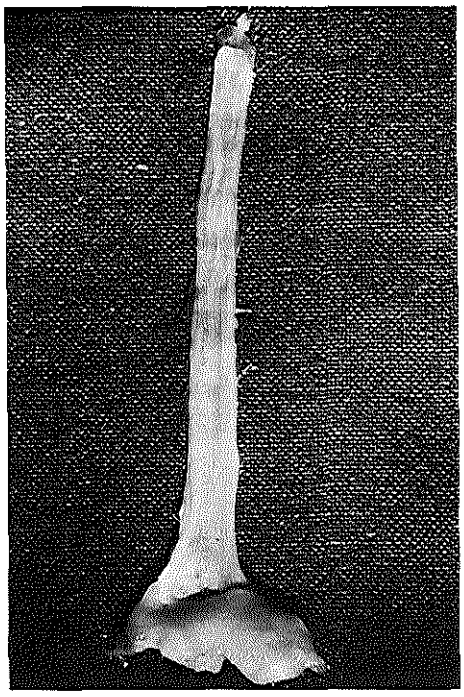


Fig. 18. An example of a normal looking oesophageal mucous membrane of a rat from group B (42 days of reflux). The oesophagus and oesophago-jejunostomy is opened longitudinally on the median ventral side.


Fig. 19. An example of an ulcerative oesophageal mucous membrane of a rat out of group M + P + G (14 days of reflux). The distal half of the oesophagus is ulcerative, in the middle part of the oesophagus linear ulcerations are visible. u = ulceration.

*Scoring of lesions*: the lesions (erosions, ulcerations and hyperkeratotic areas) were scored according to the score system described in chapter III. 7. The scores for the B, G, B + G, Roux en Y groups are given in table 5. As the scores were zero for the whole oesophagus, no further analysis was done.

Table 6 gives the scores for the other groups for the whole oesophagus. The scores of the distal part of the oesophagus are shown in table 7, for the middle part in table 8 and table 9 gives the scores of the proximal part of the oesophagus. In these tables the time in days is the vertical ordinate, the means of the scores per lesion of the sacrificed rats as the horizontal. The results are dealt with in more detail in the next chapter.

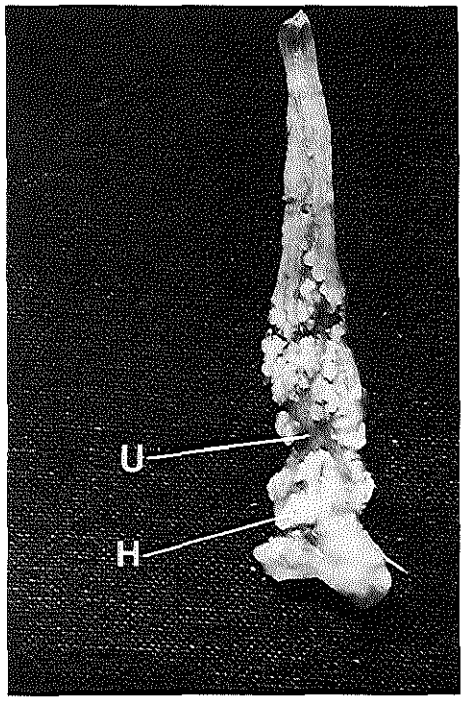



Fig. 20. An example of hyperkeratotic changes on the oesophageal mucous membrane of a rat out of group G + P (42 days of reflux). In the distal and middle part of the oesophagus, white hyperkeratotic areas between ulcerative lesions are visible. The proximal part of the oesophagus is normal. h = hyperkeratotic area, u = ulceration.

CHAPTER V

# RESULTS OF ANALYSIS OF THE OESOPHAGEAL LESIONS

## 1. Description of data as given in the tables and figures.

*Table 5* shows the mean scores of the macroscopical examination of the oesophageal mucosa of the sacrificed rats from groups B, G, B + G, and Roux en Y. In none of the rats from these groups oesophageal lesions were found.

*Table 6.* This table represents the mean scores of the lesions of the whole oesophagus in the P, P + B, P + G and P + B + G groups. Here one can see that on day 3 and 5, in particular erosive lesions are present in all groups. By the 7th post-operative day ulcerative lesions had appeared, and by day 14 hyperkeratotic patches occured, which were still present at 28 and 42 days.

*Table 7* represents the mean scores of the lesions of the distal part of the oesophagus in the P, P + B, P + G and P + B + G groups. From this table it can be seen, as in table 6, erosive lesions are notably present on day 3 and 5. From the 7th day on, ulcerative lesions are present. Hyperkeratotic patches developed from the 14th day on.

*Table 8* represents the mean scores of the lesions of the middle part of the oesophagus of the same experimental groups. The development of the various lesions is similar to the distal part of the oesophagus. However the mean scores are less than in the distal part.

*Table 9.* Here the mean scores of lesions of the proximal part of the oesophagus of the P, P + B, P + G, and P + B + G groups are shown. The scores are practically zero for erosions, ulcerations and hyperkeratotic lesions, except for day 28 and 42 where some hyperkeratotic lesions are found.

In summary: Erosions and ulcerations are first to appear. By day 14 almost no erosions are found anymore, but more extensive ulceration and hyperkeratosis is seen. This overall picture is found in all the different experimental groups.

Table 5. The means of scores of the lesions from groups B,G,B + G and Roux en Y in the whole oesophagus, of the individual rats which were sacrificed on the respective post-operative days. (nor = normal mucosa; er = erosion; ulc = ulceration; hyperk = hyperkeratosis)

| | B | | | | G | | | | B+G | | | | Roux en Y | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. |
| 3 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |
| 5 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |
| 7 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |
| 14 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |
| 28 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |
| 42 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |

Table 6. The means of scores of lesions in the whole oesophagus from groups P + B + G, P + B, P +G and P, of the individual rats which were sacrificed on the respective post-operative days.

| | P+B+G | | | | P+B | | | | P+G | | | | P | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. |
| 3 | 7.55 | 7.45 | 0 | 0 | 6.75 | 5.42 | 2.83 | 0 | 6.95 | 7.15 | 0.90 | 0 | 6.40 | 6.25 | 2.35 | 0 |
| 5 | 7.44 | 7.56 | 0 | 0 | 6.08 | 8.92 | 0 | 0 | 7.38 | 4.12 | 3.5 | 0 | 6.92 | 8.08 | 0 | 0 |
| 7 | 7.38 | 2 | 5.62 | 0 | 6.85 | 4.80 | 3.35 | 0 | 7.17 | 1.5 | 6.33 | 0 | 5.88 | 0.62 | 8.50 | 0 |
| 14 | 9.25 | 0 | 5.75 | 0 | 8.32 | 0 | 6.31 | 0.47 | 8.04 | 0 | 5.0 | 1.96 | 7.06 | 0 | 7.94 | 0 |
| 28 | 5.98 | 0 | 7.29 | 1.73 | 6.16 | 0 | 8.39 | 0.45 | 6.21 | 0 | 8.02 | 0.79 | 5.75 | 0 | 8.27 | 0.98 |
| 42 | 7.14 | 0 | 6.35 | 1.51 | 6.45 | 0 | 6.3 | 2.25 | 7.55 | 0 | 6.18 | 1.27 | 5.20 | 0.83 | 8.55 | 0.42 |

Table 7. The means of scores of the lesions in the distal part of the oesophagus from groups P + B + G, P + B, P +G and P, of the individual rats which were sacrificed on the respective post-operative days.

| | P+B+G | | | | P+B | | | | P+G | | | | P | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. |
| 3 | 0 | 5 | 0 | 0 | 0 | 3.33 | 1.67 | 0 | 0 | 4.6 | 0.4 | 0 | 0 | 4.75 | 0.25 | 0 |
| 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 2.5 | 2.5 | 0 | 0 | 5 | 0 | 0 |
| 7 | 0 | 1.25 | 3.75 | 0 | 0 | 2.8 | 2.2 | 0 | 0.22 | 1.00 | 3.78 | 0 | 0 | 0.62 | 4.38 | 0 |
| 14 | 0 | 0 | 5 | 0 | 0.25 | 0 | 4.28 | 0.47 | 0.22 | 0 | 3.12 | 1.66 | 0 | 0 | 4.31 | 0.69 |
| 28 | 0 | 0 | 4.6 | 0.4 | 0 | 0 | 4.91 | 0.09 | 0 | 0 | 4.52 | 0.48 | 0 | 0 | 4.22 | 0.78 |
| 42 | 0 | 0 | 3.65 | 1.35 | 0 | 0 | 4.05 | 0.95 | 0 | 0 | 3.79 | 1.21 | 0 | 0 | 4.58 | 0.42 |

Table 8. The means of scores of the lesions in the middle part of the oesophagus from groups P + B + G, P + G, and P, of the individual rats which were sacrificed on the respective post-operative days.

| | P+B+G | | | | P+B | | | | P+G | | | | P | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. |
| 3 | 2.55 | 2.45 | 0 | 0 | 1.75 | 2.08 | 1.17 | 0 | 1.95 | 2.55 | 0.5 | 0 | 1.65 | 1.25 | 2.10 | 0 |
| 5 | 2.44 | 2.56 | 0 | 0 | 1.08 | 3.92 | 0 | 0 | 2.38 | 1.62 | 1 | 0 | 2.17 | 2.83 | 0 | 0 |
| 7 | 2.37 | 0.75 | 1.88 | 0 | 1.85 | 2 | 1.15 | 0 | 1.95 | 0.5 | 2.55 | 0 | 1.5 | 0 | 3.5 | 0 |
| 14 | 4.25 | 0 | 0.75 | 0 | 2.96 | 0 | 2.04 | 0 | 2.01 | 0 | 1.88 | 0.31 | 1.26 | 0 | 3.62 | 0.12 |
| 28 | 0.75 | 0 | 2.69 | 1.56 | 1.24 | 0 | 3.35 | 0.36 | 1.31 | 0 | 3.38 | 0.31 | 0.75 | 0 | 4.04 | 0.21 |
| 42 | 0.62 | 0 | 2.70 | 1.68 | 1 | 0 | 2.25 | 1.75 | 1.88 | 0 | 2.26 | 0.86 | 0.45 | 0.58 | 3.97 | 0 |

Table 9. The means of scores of the lesions in the proximal part of the oesophagus from groups P + B + G, P + B, P +G, and P, of the individual rats which were sacrificed on the respective post-operative days.

| | P+B+G | | | | P+B | | | | P+G | | | | P | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. | Nor. | Er. | Ulc. | Hyperk. |
| 3 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 4.75 | 0.25 | 0 | 0 |
| 5 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 4.75 | 0.25 | 0 | 0 |
| 7 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 4.37 | 0 | 0 | 0 |
| 14 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| 28 | 5 | 0 | 0 | 0 | 4.875 | 0 | 0.125 | 0 | 4.875 | 0 | 0.125 | 0 | 5 | 0 | 0 | 0 |
| 42 | 5 | 0 | 0 | 0 | 4.33 | 0 | 0 | 0.67 | 4.875 | 0 | 0.125 | 0 | 4.75 | 0.25 | 0 | 0 |

Next the sumscores of the lesions in the oesophagus of individual rats and the mean sumscores of every small group of rats which were sacrificed at one and the same post-operative day, were calculated (see chapter III. 7). This was done to be able to compare the degree of oesophagitis at the different times post-operatively in the P, P + B, P + G and P + B + G groups. The mean ulcer sumscore, the mean erosion + ulceration sumscore, the mean erosion + ulceration + hyperkeratosis sumscore from the P, P + B, P + G, and P + B + G groups for the whole oesophagus are given in table 10. Similarly the mean sumscores of the lesions of the distal part of the oesophagus (table 11) and the middle part of the oesophagus (table 12) are given. Because the proximal part showed practically no lesions, sumscoring was not performed. The mean erosion sumscores are not given, because statistical comparison of these lesions did not appear to be relevant.

*Table 10* shows that on day 3 the mean ulceration sumscores of group P, P + B, P + G, are low, and that the mean ulceration sumscore of group P + B + G is zero. On day 5 the mean ulceration sumscore of group P + G is 3,5, which for the other groups it is zero. The mean ulcer + erosion sumscores are at these stages quite high, extensive erosive lesions being present. Because no hyperkeratotic patches are present at these stages, the mean ulcer + erosion sumscores are similar to the mean ulcer + erosion + hyperkeratotic sumscores. On day 7 and following, the mean ulcer sumscore is almost the same as the mean ulcer + erosion sumscore, practically no erosions being present. The mean ulceration + erosion sumscore is similar to the mean ulceration + erosion + hyperkeratotic sumscore, because at this time no hyperkeratotic areas are present. On day 14 the mean ulcer sumscore is almost the same as the mean ulceration + erosion sumscore, because practically only ulcerations are present. The mean ulceration + erosion + hyperkeratotic sumscore is higher than the mean ulceration + erosion sumscore, because at this time hyperkeratotic patches are present. The difference between the mean ulceration and erosion sumscore and the mean ulceration + erosion + hyperkeratotic sumscore appears greater in the later stages, because of the increase of hyperkeratosis. In table 11 and 12 the same picture is seen. No essential differences are apparant for the lesions in the distal part, the middle part of the oesophagus and the whole oesophagus. The lesions of the middle part of the oesophagus appear somewhat less extensive than the distal part.

Next with the aid of table 10, 11, 12, the mean ulcer sumscore, the mean ulcer + erosion sumscore and the mean ulcer + erosion + hyperkeratotic sumscore for the whole oesophagus, for the distal part and for the middle part of the

Table 10. The mean sumscores calculated from the sumscores of the lesions from groups P, P + B, P + G and P + B + G in the whole oesophagus, of the individual rats which were sacrificed on the respective post-operative days.

| Time days | Ulceration sumscore per rat | | | | Ulceration + erosion sumscore per rat | | | | Ulceration + erosion + hyperk. sumscore per rat | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | P+B | P+G | P+B+G | P | P+B | P+G | P+B+G | P | P+B | P+G | P+B+G | |
| 3 | 0 | 8.5 | 0 | 0 | 7.5 | 8.5 | 8.75 | 8 | 7.5 | 8.5 | 8.75 | 8 | |
| | 2.25 | 0 | 4.5 | 0 | 7.25 | 6.75 | 7.5 | 6.75 | 7.25 | 6.75 | 7.5 | 6.75 | |
| | 0 | 0 | 0 | 0 | 7 | 9.5 | 10 | 8.75 | 7 | 9.5 | 10 | 8.75 | |
| | 3.75 | | 0 | 0 | 11.25 | 9.5 | 7.75 | 6.25 | 11.25 | | 7.75 | 6.25 | |
| | 5.75 | | 0 | 0 | 10 | | 6.25 | 7.5 | 10 | | 6.25 | 7.5 | |
| | 2.35 | 2.83 | 0.9 | 0 | 8.60 | 8.25 | 8.05 | 7.45 | 8.60 | 8.25 | 8.05 | 7.45 | mean sumscore |
| 5 | 0 | 0 | 7 | 0 | 6 | 7.5 | 7 | 7.5 | 6 | 7.5 | 7 | 7.5 | |
| | 0 | 0 | 0 | 0 | 10.75 | 9.75 | 8.25 | 8 | 10.75 | 9.75 | 8.25 | 8 | |
| | 0 | 0 | | 0 | 7.5 | 9.5 | | 6.25 | 7.5 | 9.5 | | 6.25 | |
| | | | | 0 | | | | 8.5 | | | | 8.5 | |
| | 0 | 0 | 3.5 | 0 | 8.08 | 8.92 | 7.62 | 7.56 | 8.08 | 8.92 | 7.62 | 7.56 | mean sumscore |
| 7 | 9 | 0 | 6.7 | 8.75 | 9 | 8.5 | 6.7 | 8.75 | 9 | 8.5 | 6.7 | 8.75 | |
| | 5.25 | 0 | 9.5 | 6.25 | 7.75 | 8 | 9.5 | 6.25 | 7.75 | 8 | 9.5 | 6.25 | |
| | 11.25 | 7.5 | 7.45 | 7.5 | 11.25 | 7.5 | 7.45 | 7.5 | 11.25 | 7.5 | 7.45 | 7.5 | |
| | 8.5 | 8 | 8 | 0 | 8.5 | 8 | 8 | 8 | 8.5 | 8 | 8 | 8 | |
| | | 1.25 | 0 | | | 8.75 | 7.5 | | | 8.75 | 7.5 | | |
| | 8.5 | 3.35 | 6.33 | 5.62 | 9.12 | 8.15 | 7.83 | 7.62 | 9.12 | 8.15 | 7.83 | 7.62 | mean sumscore |
| 14 | 8.35 | 4.75 | 3.75 | 6.5 | 8.35 | 4.75 | 3.75 | 6.5 | 8.5 | 6.25 | 7.5 | 6.5 | |
| | 6.9 | 5.85 | 4.45 | 5 | 6.9 | 5.85 | 4.45 | 5 | 8.85 | 6.25 | 6.25 | 5 | |
| | 9.5 | 6.9 | 5.5 | | 9.5 | 6.9 | 5.5 | | 10 | 6.9 | 7.5 | | |
| | 7 | 7.75 | 6.3 | | 7 | 7.75 | 6.3 | | 7.6 | 7.75 | 6.6 | | |
| | 7.94 | 6.31 | 5.0 | 5.75 | 7.94 | 6.31 | 5.0 | 5.75 | 8.74 | 6.78 | 6.96 | 5.75 | mean sumscore |
| 28 | 8.55 | 9.4 | 7.45 | 9.85 | 8.55 | 9.4 | 7.45 | 9.85 | 10 | 9.5 | 7.75 | 10 | |
| | 7.35 | 8.15 | 7.2 | 6.85 | 7.35 | 8.15 | 7.2 | 6.85 | 8.25 | 8.35 | 9 | 10 | |
| | 8.9 | 6.9 | 7.25 | 7 | 8.9 | 6.9 | 7.25 | 7 | 9.5 | 7 | 8 | 9.25 | |
| | | 9.3 | 10.2 | 5.45 | | 9.1 | 10.2 | 5.45 | | 10.5 | 10.5 | 7.75 | |
| | 8.27 | 8.39 | 8.02 | 7.29 | 8.27 | 8.39 | 8.02 | 7.29 | 9.25 | 8.84 | 8.81 | 9.25 | mean sumscore |
| 42 | 9.4 | 7.15 | 8.2 | 3.9 | 9.4 | 7.15 | 8.2 | 3.9 | 9.9 | 8.25 | 9 | 9.75 | |
| | 10 | 3 | 4.9 | 8.8 | 10 | 3 | 4.9 | 8.8 | 10 | 12 | 9 | 9 | |
| | 6.25 | 8.75 | 5.1 | | 8.75 | 8.75 | 5.1 | | 9.5 | 8.75 | 8 | | |
| | | | 6.5 | | | | 6.5 | | | | 7 | | |
| | 8.55 | 6.30 | 6.18 | 6.35 | 9.32 | 6.30 | 6.18 | 6.35 | 9.80 | 9.67 | 8.25 | 9.38 | mean sumscore |

Table 11. The mean sumscores calculated from the sumscores of the lesions from groups P, P + B, P + G, and P + B + G in the distal part of the oesophagus, of the individual rats which were sacrificed on the respective post-operative days.

| Time days | Ulceration sumscore per rat | | | | Ulceration + erosion sumscore per rat | | | | Ulceration + erosion + hyperk. sumscore per rat | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | P+B | P+G | P+B+G | P | P+B | P+G | P+B+G | P | P+B | P+G | P+B+G | |
| 3 | 0 | 5 | 0 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | 0 | 0 | 2 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | 0 | 0 | 0 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | 0 | | 0 | 0 | 5 | | 5 | 5 | 5 | | 5 | 5 | |
| | 1,25 | | 0 | 0 | 5 | | 5 | 5 | 5 | | 5 | 5 | |
| | 0,25 | 1,67 | 0,4 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | mean sumscore |
| 5 | 0 | 0 | 5 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | 0 | 0 | 0 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | 0 | 0 | | 0 | 5 | 5 | | 5 | 5 | 5 | | 5 | |
| | | | | 0 | | | | 5 | | | | 5 | |
| | 0 | 0 | 2,5 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | mean sumscore |
| 7 | 5 | 0 | 4,7 | 5 | 5 | 5 | 4,7 | 5 | 5 | 5 | 4,7 | 5 | |
| | 2,5 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | 5 | 5 | 4,2 | 5 | 5 | 5 | 4,2 | 5 | 5 | 5 | 4,2 | 5 | |
| | 5 | 5 | 5 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | |
| | | 1 | 0 | | | 5 | 5 | | | 5 | 5 | | |
| | 4,38 | 2,2 | 3,78 | 3,75 | 5 | 5 | 4,78 | 5 | 5 | 5 | 4,78 | 5 | mean sumscore |
| 14 | 4,85 | 3,5 | 2,5 | 5 | 4,85 | 3,5 | 2,5 | 5 | 5 | 5 | 5 | 5 | |
| | 3,4 | 4,6 | 3,2 | 5 | 3,4 | 4,6 | 3,2 | 5 | 5 | 5 | 5 | 5 | |
| | 4,5 | 5 | 3 | | 4,5 | 5 | 3 | | 5 | 5 | 5 | | |
| | 4,5 | 4 | 3,8 | | 4,5 | 4 | 3,8 | | 5 | 4 | 4,1 | | |
| | 4,31 | 4,28 | 3,12 | 5 | 4,31 | 4,28 | 3,12 | 5 | 5 | 4,75 | 4,78 | 5 | mean sumscore |
| 28 | 4,15 | 4,9 | 4,7 | 4,85 | 4,15 | 4,9 | 4,7 | 4,85 | 5 | 5 | 5 | 5 | |
| | 4,1 | 5 | 4,2 | 4,85 | 4,1 | 5 | 4,2 | 4,85 | 5 | 5 | 5 | 5 | |
| | 4,4 | 4,9 | 4,5 | 4 | 4,4 | 4,9 | 4,5 | 4 | 5 | 5 | 5 | 5 | |
| | | 4,85 | 4,7 | 4,7 | | 4,85 | 4,7 | 4,7 | | 5 | 5 | 5 | |
| | 4,22 | 4,91 | 4,52 | 4,60 | 4,22 | 4,91 | 4,52 | 4,60 | 5 | 5 | 5 | 5 | mean sumscore |
| 42 | 4,5 | 4,65 | 4,65 | 2,5 | 4,5 | 4,65 | 4,65 | 2,5 | 5 | 5 | 5 | 5 | |
| | 5 | 2,5 | 1,4 | 4,8 | 5 | 2,5 | 1,4 | 4,8 | 5 | 5 | 5 | 5 | |
| | 4,25 | 5 | 4,6 | | 4,25 | 5 | 4,6 | | 5 | 5 | 5 | | |
| | | | 4,5 | | | | 4,5 | | | | 5 | | |
| | 4,58 | 4,05 | 3,79 | 3,65 | 4,58 | 4,05 | 3,79 | 3,65 | 5 | 5 | 5 | 5 | mean sumscore |

Table 12. The mean sumscores calculated from the sumscores of the lesions from groups P, P + B, P + G, and P + B + G in the middle part of the oesophagus, of the individual rats which were sacrificed on the respective post-operative days.

| Time days | Ulceration sumscore per rat | | | | Ulceration + erosion sumscore per rat | | | | Ulceration + erosion + hyperk. sumscore per rat | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | P+B | P+G | P+B+G | P | P+B | P+G | P+B+G | P | P+B | P+G | P+B+G | |
| 3 | 0 | 3.5 | 0 | 0 | 2.5 | 3.5 | 3.75 | 3 | 0.5 | 3.5 | 3.75 | 3 | |
| | 3.25 | 0 | 2.5 | 0 | 2.25 | 1.75 | 2.5 | 1.75 | 2.25 | 1.75 | 2.5 | 1.75 | |
| | 0 | 0 | 0 | 0 | 2 | 4.5 | 5 | 3.75 | 2 | 4.5 | 5 | 3.75 | |
| | 3.75 | | 0 | 0 | 5 | | 2.75 | 1.25 | 5 | | 2.75 | 1.25 | |
| | 4.5 | | 0 | 0 | 5 | | 1.25 | 2.5 | 5 | | 1.25 | 2.5 | |
| | 2.10 | 1.17 | 0.5 | 0 | 3.35 | 3.25 | 3.05 | 2.45 | 3.35 | 3.25 | 3.05 | 2.45 | mean sumscore |
| 5 | 0 | 0 | 2 | 0 | 1 | 2.5 | 2 | 2.5 | 1 | 2.5 | 2 | 2.5 | |
| | 0 | 0 | 0 | 0 | 5 | 4.75 | 3.25 | 3 | 5 | 4.75 | 3.25 | 3 | |
| | 0 | 0 | | 0 | 2.5 | 4.5 | | 1.25 | 2.5 | 4.5 | | 1.25 | |
| | | | | 0 | | | | 3.5 | | | | 3.5 | |
| | 0 | 0 | 1 | 0 | 2.83 | 3.92 | 2.62 | 2.56 | 2.83 | 3.92 | 2.62 | 2.56 | mean sumscore |
| 7 | 4 | 0 | 2 | 3.75 | 4 | 3.5 | 2 | 3.75 | 4 | 3.5 | 2 | 3.75 | |
| | 2.75 | 0 | 4.5 | 1.25 | 2.75 | 3 | 4.5 | 1.25 | 2.75 | 3 | 4.5 | 1.25 | |
| | 3.75 | 3.5 | 3.25 | 2.5 | 3.75 | 2.5 | 3.25 | 2.5 | 3.75 | 2.5 | 3.25 | 2.5 | |
| | 3.5 | 3 | 3 | 0 | 3.5 | 3 | 3 | 3 | 3.5 | 3 | 3 | 3 | |
| | | 0.25 | 0 | | | 3.75 | 2.5 | | | 3.75 | 2.5 | | |
| | 3.50 | 1.15 | 2.55 | 1.88 | 3.50 | 3.15 | 3.05 | 2.63 | 3.50 | 3.15 | 3.05 | 2.63 | mean sumscore |
| 14 | 3.5 | 1.25 | 1.25 | 1.5 | 3.5 | 1.25 | 1.25 | 1.5 | 3.5 | 1.25 | 2.5 | 1.5 | |
| | 3.5 | 1.25 | 1.25 | 0 | 3.5 | 1.25 | 1.25 | 0 | 3.85 | 1.25 | 1.25 | 0 | |
| | 2 | 1.9 | 2.5 | | 5 | 1.9 | 2.5 | | 5 | 1.9 | 2.5 | | |
| | 2.5 | 3.75 | 2.5 | | 2.5 | 3.75 | 2.5 | | 2.6 | 3.75 | 2.5 | | |
| | 3.62 | 2.04 | 1.88 | 0.75 | 3.62 | 2.04 | 1.88 | 0.75 | 3.74 | 2.04 | 2.19 | 0.75 | mean sumscore |
| 28 | 4.4 | 4.5 | 2.75 | 5 | 4.4 | 4.5 | 2.75 | 5 | 5 | 4.5 | 2.75 | 5 | |
| | 3.25 | 3.15 | 3 | 2 | 3.25 | 3.15 | 3 | 2 | 3.25 | 3.35 | 4 | 5 | |
| | 4.5 | 2 | 2.75 | 3 | 4.5 | 2 | 2.75 | 3 | 4.5 | 2 | 3 | 4.25 | |
| | | 3.75 | 5 | 0.75 | | 3.75 | 5 | 0.75 | | 5 | 5 | 2.75 | |
| | 4.04 | 3.35 | 3.38 | 2.69 | 4.04 | 3.35 | 3.38 | 2.69 | 4.25 | 3.71 | 3.69 | 4.25 | mean sumscore |
| 42 | 4.9 | 2.5 | 3.55 | 1.40 | 4.9 | 2.5 | 3.55 | 1.4 | 4.9 | 3.25 | 4 | 4.75 | |
| | 5 | 0.5 | 3 | 4 | 4 | 0.5 | 3 | 4 | 5 | 5 | 3.5 | 4 | |
| | 2 | 3.75 | 0.5 | | 3.75 | 3.75 | 0.5 | | 3.75 | 3.75 | 3 | | |
| | | | 2 | | | | 2 | | | | 2 | | |
| | 3.97 | 2.25 | 2.26 | 2.70 | 4.55 | 2.25 | 2.26 | 2.70 | 4.55 | 4.00 | 3.12 | 4.38 | mean sumscore |

oesophagus, were graphically represented with respect to time in days. Only the graphs of the mean sumscores for the whole oesophagus are represented and dealt with separately.

*Figure 21* is the graphical representation of the mean ulceration sumscores for the whole oesophagus with respect to time. The mean sumscore graphs of group P + B and group G + P + B show on day 3 and 5 a spiking course. This is to say that on day 3 ulcerative lesions are present which disappear on day 7. Difficulty in discrimination between ulcerations and erosions in the initial phase is responsible for this picture. The graphs of groups G, B, G + B and Roux en Y show no macroscopic lesions (mean sumscore 0). The P-group appears to have the highest sumscore in alle stages.

*Figure 22* is the graphical representation of the mean ulceration + erosion sumscores for the whole oesophagus. Because the mean ulceration + mean erosion sumscores are added, there is no spiking course of the mean sumscore curves visible in the initial phase. The P-group has the highest mean sumscores in almost all stages, except on day 28 where the mean sumscore of the P + B group is higher. The graphs of groups G, B, G + B and Roux en Y show no macroscopic lesions (mean sumscore 0).

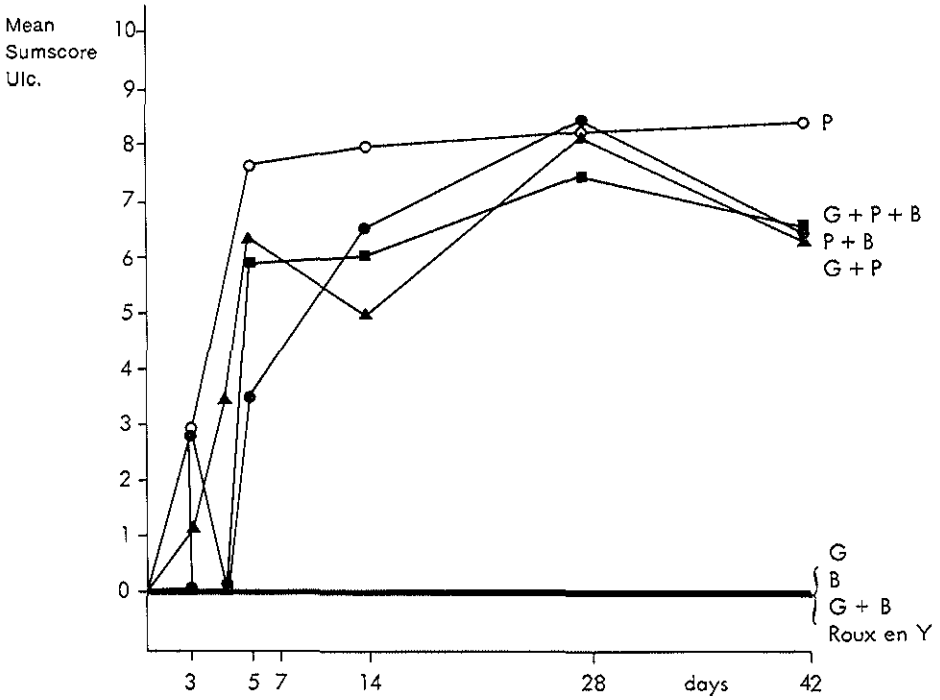



Fig. 21. Graphical representation of the mean ulceration sumscores in the whole oesophagus, from groups P + B + G, P + B, P + G, and P with respect to time in days.

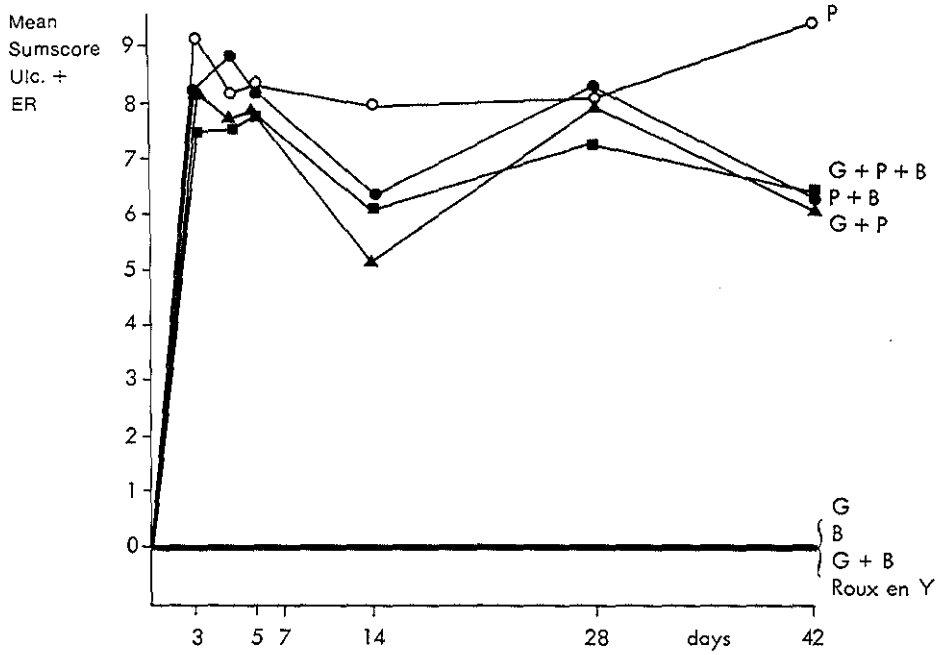



Fig. 22. Graphical representation of mean ulceration + erosion sumscores in the whole oesophagus from groups P + B + G, P + B, P + G, and P, with respect to time in days.

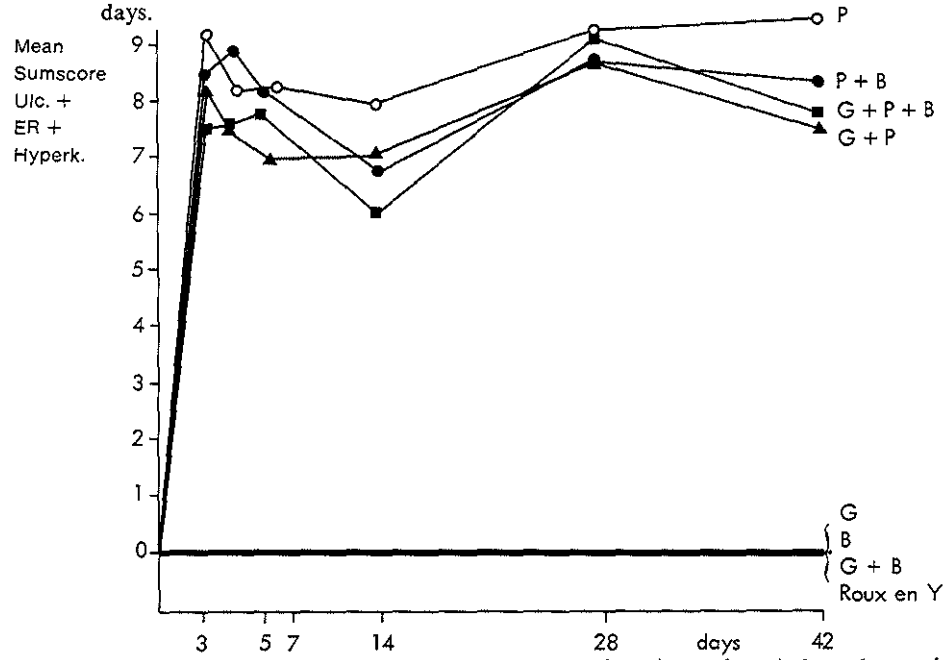



Fig. 23. Graphical representation of the mean ulceration + erosion + hyperkeratosis sumscores in the whole oesophagus from groups P + B + G, P + B, P + G, and P with respect to time in days.

*Figure 23* is the graphical representation of the mean ulceration + erosion + hyperkeratotic sumscores for the whole oesophagus, in the respective postoperative day. The P-group here also has the highest mean sumscore in almost all stages. On day 28 and 42 the mean ulceration + erosion + hyperkeratotic sumsocres are higher than the mean ulceration + erosion sumscores as given in fig. 3.2, because in these stages hyperkeratosis is also present. The graphs of groups G, B, G + B and Roux en Y again show no macroscopic lesions (sumscore 0). On the other hand the P-group appears to have the highest sumscore in all stages, except on day 5 where the mean sumscore of the P + B group is higher.

## 2. Statistical analysis of the oesophageal lesions.

### *2.1. Introduction.*

After scoring of the lesions, calculation of the sumscores and the mean sumscores, and graphical presentation of the mean sumscores against time in days, the results were analysed statistically. The scores were semi-quantitative. In order to avoid biases in the assessment of the lesions, randomisation was employed to validly test for possible differences in the experimental groups. The data presented shows that there is without a doubt a clear difference between the groups P, P + B, P + G and P + B + G, and the groups B, B + G, G and the Roux en Y group. The latter groups demonstrated no macroscopical lesions at all (see table 5). Needless to say, only groups in which abnormalities were found were compared. A close statistical comparison was therefore carried out on the first four named groups only (see table 10, 11, 12).

The overal-differences between the time curves of the mean ulceration + erosion + hyperkeratosis sumscore, the mean ulceration + erosion sumscore and the mean ulceration sumscore of the whole of the distal part and the middle part of the oesophagus were analysed by means of the Friedman test. The mean erosion sumscore time curves were not analysed, because erosions appeared only in the early post-operative period.

The mean sumscores were replaced by rank numbers (1, 2, 3 and 4). These were added (S = sum of the rank numbers), and thereafter the difference of expectation of the sum of the numbers (E) was determined under the null-hypothesis. When the null-hypothesis is correct (that is to say when *there is no* systematic difference between the time curves), then the difference between S and E is small. Accordingly the test criterium K was calculated by squaring the differences, and adding them. If K was large, the null-hypothesis

could be rejected. By means of the Friedman test table the significance probability (P) was worked out from the test criterium K.

*2.2. Analysis of the overal-differences between the four time curves of the whole oesophagus.*

Table 13. The *whole* oesophagus: mean ulceration + erosion + hyperk. sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 4 | 3 | 2 | 1 | |
| 5 | 3 | 4 | 2 | 1 | |
| 7 | 4 | 3 | 2 | 1 | |
| 14 | 4 | 2 | 3 | 1 | |
| 28 | 3,5 | 2 | 1 | 3,5 | |
| 42 | 4 | 3 | 1 | 2 | |
| S | 22,5 | 17 | 11 | 9,5 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 7,5 | 2 | -4 | -5,5 | 0 |
| $(S-E)^2$ | 56,25 | 4 | 16 | 30,25 | K = 106,5 |
| | | | | | P < 0.01 |

*Conclusion*: There are significant overal-differences between the four groups, for the mean ulceration + erosion + hyperkeratosis sumscore of the whole oesophagus ($P < 0.01$).

Table 14. The *whole* oesophagus: mean ulceration + erosion sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 4 | 3 | 2 | 1 | |
| 5 | 3 | 4 | 2 | 1 | |
| 7 | 4 | 3 | 2 | 1 | |
| 14 | 4 | 3 | 1 | 2 | |
| 28 | 3 | 4 | 2 | 1 | |
| 42 | 4 | 2 | 1 | 3 | |
| S | 22 | 19 | 10 | 9 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 7 | 4 | -5 | -6 | 0 |
| $(S-E)^2$ | 49 | 16 | 25 | 36 | K = 126 |
| | | | | | P < 0,01 |

*Conclusion*: There are significant overal-differences between the four groups, for the mean ulceration + erosion sumscore of the whole oesophagus ($P < 0.01$).

Table 15. The *whole* oesophagus: mean ulceration sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 3 | 4 | 2 | 1 | |
| 5 | 2 | 2 | 4 | 2 | |
| 7 | 4 | 1 | 3 | 2 | |
| 14 | 4 | 3 | 1 | 2 | |
| 28 | 3 | 4 | 2 | 1 | |
| 42 | 4 | 2 | 1 | 3 | |
| S | 20 | 16 | 13 | 11 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 5 | 1 | -2 | -4 | 0 |
| $(S-E)^2$ | 25 | 1 | -4 | 16 | K = 46 |
| | | | | | P > 0,05 |

*Conclusion*: There are no overal-differences between the four groups for the mean ulceration sumscore of the whole oesophagus ($P > 0.05$).

## 2.3. *Analysis of the overal-differences between the four time curves of the distal part of the oesophagus.*

Table 16. The *distal* part of the oesophagus: mean ulceration + erosion + hyperk. sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 2,5 | 2,5 | 2,5 | 2,5 | |
| 5 | 2,5 | 2,5 | 2,5 | 2,5 | |
| 7 | 3 | 3 | 1 | 3 | |
| 14 | 3 | 2 | 1 | 4 | |
| 28 | 1 | 4 | 2 | 3 | |
| 42 | 4 | 3 | 2 | 1 | |
| S | 16 | 17 | 11 | 16 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 1 | 2 | -4 | 1 | 0 |
| $(S-E)^2$ | 1 | 4 | 16 | 1 | K = 22 |
| | | | | | P > 0,05 |

*Conclusion*: There are no overal-differences between the four groups for the mean ulceration + erosion + hyperkeratosis sumscore of the distal part of the oesophagus ($P > 0.05$).

Table 17. The *distal* part of the oesophagus: mean ulceration + erosion sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 2,5 | 2,5 | 2,5 | 2,5 | |
| 5 | 2,5 | 2,5 | 2,5 | 2,5 | |
| 7 | 3 | 3 | 1 | 3 | |
| 14 | 3 | 2 | 1 | 4 | |
| 28 | 1 | 4 | 2 | 3 | |
| 42 | 4 | 3 | 2 | 1 | |
| S | 16 | 17 | 11 | 16 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 1 | 2 | -4 | 1 | 0 |
| $(S-E)^2$ | 1 | 4 | 16 | 1 | K = 22 |
| | | | | | P > 0,05 |

*Conclusion*: There are no overal-differences between the four groups for the mean ulceration + erosion sumscore of the distal part of the oesophagus ($P > 0.05$).

Table 18. The *distal* part of the oesophagus: mean ulceration sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 2 | 4 | 3 | 1 | |
| 5 | 2 | 2 | 4 | 2 | |
| 7 | 4 | 1 | 3 | 2 | |
| 14 | 3 | 2 | 1 | 4 | |
| 28 | 1 | 4 | 2 | 3 | |
| 42 | 4 | 3 | 2 | 1 | |
| S | 16 | 16 | 15 | 13 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 1 | 1 | 0 | -2 | 0 |
| $(S-E)^2$ | 1 | 1 | 0 | 4 | K = 6 |
| | | | | | P > 0,05 |

*Conclusion:* There are no overal-differences between the four groups for the mean ulceration sumscore of the distal part of the oesophagus ($P > 0.05$).

## 2.4. *Analysis of the overal-differences between the four time curves of the middle part of the oesophagus.*

Table 19. The *middle* part of the oesophagus: mean ulceration + erosion + hyperk. sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 4 | 3 | 2 | 1 | |
| 5 | 3 | 4 | 2 | 1 | |
| 7 | 4 | 3 | 2 | 1 | |
| 14 | 4 | 2 | 3 | 1 | |
| 28 | 3,5 | 2 | 1 | 3,5 | |
| 42 | 4 | 2 | 1 | 3 | |
| S | 22,5 | 16 | 11 | 10,5 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 7,5 | 1 | -4 | -4,5 | 0 |
| $(S-E)^2$ | 56,25 | 1 | 16 | 20,25 | K = 93,5 |
| | | | | | P < 0,05 |

*Conclusion*: There are overal-differences between the four groups for the mean ulceration + erosion + hyperkeratosis sumscore of the middle part of the oesophagus ($P < 0.05$).

Table 20. The *middle* part of the oesophagus: mean ulceration + erosion sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 4 | 3 | 2 | 1 | |
| 5 | 3 | 4 | 2 | 1 | |
| 7 | 4 | 3 | 2 | 1 | |
| 14 | 4 | 3 | 2 | 1 | |
| 28 | 4 | 2 | 3 | 1 | |
| 42 | 4 | 1 | 2 | 3 | |
| S | 23 | 16 | 13 | 8 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 8 | 1 | -2 | -7 | 0 |
| $(S-E)^2$ | 64 | 1 | 4 | 49 | K = 118 |
| | | | | | P < 0,01 |

*Conclusion*: There are overal-differences between the four groups for the mean ulceration + erosion sumscore of the middle part of the oesophagus ($P < 0.01$).

Table 21: The *middle* part of the oesophagus: mean ulceration sumscore.

| Time | P | P+B | P+G | P+B+G | |
|---|---|---|---|---|---|
| 3 | 4 | 3 | 2 | 1 | |
| 5 | 2 | 2 | 4 | 2 | |
| 7 | 4 | 1 | 3 | 2 | |
| 14 | 4 | 3 | 2 | 1 | |
| 28 | 4 | 2 | 3 | 1 | |
| 42 | 4 | 1 | 2 | 3 | |
| S | 22 | 12 | 16 | 10 | 60 |
| E | 15 | 15 | 15 | 15 | 60 |
| S-E | 7 | -3 | 1 | -5 | 0 |
| $(S-E)^2$ | 49 | 9 | 1 | 25 | K = 84 |
| | | | | | P < 0,05 |

*Conclusion*: There are overal-differences between the four groups for the mean ulceration sumscore of the middle part of the oesophagus ($P < 0.05$).

*2.5. Summary.*

When the Friedman test was used in comparing the P, P + B, P + G and P + B + G groups, there were significant overal-differences between the four groups for the mean ulceration + erosion + hyperkeratosis sumscore and for the mean ulceration + erosion sumscore of the whole oesophagus. Also significant differences were found for all mean sumscores of the middle part of the oesophagus. There were no significant differences for the mean sumscores of the lesions in the distal part of the oesophagus. These results show that when all the lesions were taken together, the destruction of the oesophagus by pancreatic juice alone, was more serious than when reflux of pancreatic juice together with bile and/or gastric juice occured. The differences between the group with reflux of pancreatic juice alone and the other groups with reflux of combinations of pancreatic juice were further analysed statistically. Bile and gastric juice both appeared to lessen the "destructive" effect of pancreatic juice.

## 3 Further analysis of the overal-differences of the four time curves.

*3.1. Introduction.*

After testing the overal differences between the time curves, further testing of the overal-differences was carried out to determine whether the addition of

bile or gastric juice to pancreatic juice, resulted in a significant change in the destructive effect of the pancreatic juice on oesophageal mucosa.
The sign test was used to evaluate the mean ulceration + erosion + hyperkeratosis sumscore and the mean ulceration + erosion sumscore, for the whole and the middle part of the oesophagus, and the mean ulceration sumscore of the middle part of the oesophagus. This further test on the overal-differences between the mean ulceration sumscore of the whole oesophagus and on all mean sumscores of the distal part of the oesophagus was not performed, since according to the Friedman test no signifcant differences between the time curves was found, sothat no closer analysis of the individual time curves was carried out. Gastric juice and bile neither potentiated nor diminished the "destructive" effect of pancreatic juice in the distal oesophagus. In the proximal oesophagus virtually no lesions were found, so that no analysis was performed here either.

### *3.2. Further analysis of the overal-differences of the four time curves of the whole oesophagus.*

*Effect of bile only*: The differences between group P + B and group P and between group P + B + G and group P + G was tested by means of the sign test. This was considered valid, since further statistical analysis employing the test for interaction had shown the effect of gastric juice was not affected by the presence of bile.

Table 22: The whole oesophagus: mean ulceration + erosion + hyperkeratosis sumscore.

| (P+B) | - | (P) | | (P+B+G) | - | (P+G) | | |
|---|---|---|---|---|---|---|---|---|
| 3 | | 4 | - | 1 | | 2 | - | |
| 4 | | 3 | + | 1 | | 2 | - | |
| 3 | | 4 | - | 1 | | 2 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 2 | | 3,5 | - | 3,5 | | 1 | + | |
| 3 | | 4 | - | 2 | | 1 | + | |

*Results*: 12 differences: 3 positive and 9 negative.

*Conclusion*: Bile has apparently a slight inhibitory effect on the "destructive" effect of pancreatic juice. The difference is not shown to be significant by the sign-test ($P > 0.10$)

Table 23. *The whole oesophagus*: mean ulceration + erosion + hyperkeratosis sumscore. *Effect of gastric juice only*: Tested on the differences between the P + G and P and P + B + G and P + B groups.

| (P+G) | | | (P+B+G) | | | |
|---|---|---|---|---|---|---|
| 2 | 4 | - | 1 | 3 | - | |
| 2 | 3 | - | 1 | 4 | - | |
| 2 | 4 | - | 1 | 3 | - | |
| 3 | 4 | - | 1 | 2 | - | |
| 1 | 3,5 | - | 3,5 | 2 | + | |
| 1 | 4 | - | 2 | 3 | - | |

*Results:* 12 differences: 11 negative and 1 positive.
*Conclusion*: Gastric juice has a significant inhibitory effect on the "destructive" effect of pancreatic juice ($P < 0.01$).

Table 24. *The whole oesophagus*: mean ulceration + erosion sumscore. *Effect of bile only*: Tested on the differences between the P + B and P and P + B + G and P + G groups.

| (P+B) | | | (P+B+G) | | | |
|---|---|---|---|---|---|---|
| 3 | 4 | - | 1 | 2 | - | |
| 4 | 3 | + | 1 | 2 | - | |
| 3 | 4 | - | 1 | 2 | - | |
| 3 | 4 | - | 2 | 1 | + | |
| 4 | 3 | + | 1 | 2 | - | |
| 2 | 4 | - | 3 | 1 | + | |

*Results:* 12 differences: 4 positive and 8 negative.
*Conclusion*: An inhibitory effect of bile on the "destructive" effect of pancreatic juice is not demonstrable ($P > 0.10$).

Table 25. *The whole oesophagus*: mean ulceration + erosion sumscore.
*Effect of gastric juice only*: tested on the differences between P + G and P and P + B + G and P + B groups.

| (P+G) | (P) | | (P+B+G) | (P+B) | | |
|---|---|---|---|---|---|---|
| 2 | 4 | - | 1 | 3 | - | |
| 2 | 3 | - | 1 | 4 | - | |
| 2 | 4 | - | 1 | 3 | - | |
| 1 | 4 | - | 2 | 3 | - | |
| 2 | 3 | - | 1 | 4 | - | |
| 1 | 4 | - | 3 | 2 | + | |

*Results:* 12 differences: 11 negative and 1 positive.
*Conclusion*: Gastric juice has a significant inhibitory effect on the "destructive" effect of pancreatic juice ($P < 0.01$).

### 3.3. *Further analysis of the overal-differences of the four time curves of the middle part of the oesophagus.*

Table 26. *The middle part of the oesophagus*: mean ulceration + erosion + hyperkeratosis sumscore.
*Effect of bile only*: tested on the differences between P + B and P and P + B + G and P + G groups.

| (P+B) | (P) | | (P+B+G) | (P+G) | | |
|---|---|---|---|---|---|---|
| 3 | 4 | - | 1 | 2 | - | |
| 4 | 3 | + | 1 | 2 | - | |
| 3 | 4 | - | 1 | 2 | - | |
| 2 | 4 | - | 1 | 3 | - | |
| 2 | 3,5 | - | 3,5 | 1 | + | |
| 2 | 4 | - | 3 | 1 | + | |

*Results:* 12 differences: 9 negative and 3 positive.
*Conclusion*: Bile has apparently a slight inhibitory effect on the "destructive" effect of pancreatic juice. This difference is not shown to be significant by the sign test ($P > 0.10$).

Table 27. *The middle part of the oesophagus*: mean ulceration + erosion + hyperkeratosis sumscore.
*Effect of gastric juice only*: tested on the differences between P + G and P and P + B + G and P + B groups.

| (P+G) | - | (P) | | (P+B+G) | - | (P+B) | | |
|---|---|---|---|---|---|---|---|---|
| 2 | | 4 | - | 1 | | 3 | - | |
| 2 | | 3 | - | 1 | | 4 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 3 | | 4 | - | 1 | | 2 | - | |
| 2 | | 4 | - | 3 | | 1 | + | |

*Results:* 12 differences: 11 negative and 1 positive.
*Conclusion*: Gastric juice has a significant inhibitory effect on the "destructive" effect of pancreatic juice ($P < 0.01$).

Table 28. *The middle part of the oesophagus*: mean ulceration + erosion sumscore
*Effect of bile only*: tested on the differences between the P + B and P and P + B + G and P + G groups.

| (P+B) | - | (P) | | (P+B+G) | - | (P+G) | | |
|---|---|---|---|---|---|---|---|---|
| 3 | | 4 | - | 1 | | 2 | - | |
| 4 | | 3 | + | 1 | | 2 | - | |
| 3 | | 4 | - | 1 | | 2 | - | |
| 3 | | 4 | - | 1 | | 2 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 1 | | 4 | - | 3 | | 2 | + | |

*Results:* 12 differences: 10 negative and 2 positive.
*Conclusion*: Bile has a significant inhibitory effect on the "destructive" effect of pancreatic juice ($P < 0.05$).

Table 29. *The middle part of the oesophagus*: mean ulceration + erosion sumscore.
*Effect of gastric juice only*: tested on the differences between the P + G and P + P + B + G and P + B groups.

| (P+G) | - | (P) | | (P+B+G) | - | (P+B) | | |
|---|---|---|---|---|---|---|---|---|
| 2 | | 4 | - | 1 | | 3 | - | |
| 2 | | 3 | - | 1 | | 4 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 3 | | 4 | - | 1 | | 2 | - | |
| 2 | | 4 | - | 3 | | 1 | + | |

*Results:* 12 differences: 11 negative and 1 positive.
*Conclusion*: Gastric juice has a significant inhibitory effect on the "destructive" effect of pancreatic juice ($P < 0.01$).

Table 30. *The middle part of the oesophagus*: mean ulceration sumscore.
Effect of bile only: tested on the differences between P + B and P and P + B + G and P + G groups.

| (P+B) | - | (P) | | (P+B+G) | - | (P+G) | | |
|---|---|---|---|---|---|---|---|---|
| 3 | | 4 | - | 1 | | 2 | - | |
| 2 | | 2 | 0 | 2 | | 4 | - | |
| 1 | | 4 | - | 2 | | 3 | - | |
| 3 | | 4 | - | 1 | | 2 | - | |
| 2 | | 4 | - | 1 | | 3 | - | |
| 1 | | 4 | - | 3 | | 2 | + | |

*Results:* 11 differences: 10 negative and 1 positive.
*Conclusion*: Bile has a significant inhibitory effect on the "destructive" effect of pancreatic juice ($P < 0.02$).

Table 31. *The middle part of the oesophagus*: mean ulceration sumscore.
*The effect of gastric juice only*: tested on the difference between the P + G and P and P + B + G and P + B groups.

| (P+G) | (P) | | (P+B+G) | (P+B) | | |
|---|---|---|---|---|---|---|
| 2 | 4 | - | 1 | 3 | - | |
| 4 | 2 | + | 2 | 2 | 0 | |
| 3 | 4 | - | 2 | 1 | + | |
| 2 | 4 | - | 1 | 3 | - | |
| 3 | 4 | - | 1 | 2 | - | |
| 2 | 4 | - | 3 | 1 | + | |

*Results:* 11 differences: 3 positive and 8 negative.
*Conclusion*: Gastric juice has no significant inhibitory effect on the "destructive" effect of pancreatic juice ($P > 0.10$).

### *3.4. Summary.*

With the use of the sign-test, the overal-differences found between the lesions in the P, P + B, P + G, and P + B + G groups were further analysed. The results showed that gastric juice had a significant inhibitory effect on the extent of the lesions produced by pancreatic juice. This was shown by comparison of the mean ulceration + erosion sumscore and the mean ulceration + erosion + hyperkeratosis sumscores of the whole and the middle part of the oesophagus ($P < 0.01$).
No significant inhibitory or potentiating effect by gastric juice could be shown when the mean sumscores of the distal part of the oesophagus were compared.
Bile showed a statistically significant inhibitory effect on the "destructive" effect of pancreatic juice, when the mean ulceration + erosion sumscore ($P < 0.05$) and the mean ulceration sumscore ($p < 0.02$) of the middle part of the oesophagus were compared.
For the mean sumscores of the whole oesophagus or the distal part of the oesophagus, no inhibitory effect of bile could be found.

## 4. Conclusions.

From the results of statistical evaluation of all the experimental groups it was clear that only reflux of pancreatic juice alone or in combination with bile and/or gastric juice produced lesions in the oesophagus.
The ulcerative lesions in the whole oesophagus were equally extensive in all the groups. This was true also for the ulcerations in the distal part of the oesophagus. In the midle part of the oesophagus however, ulceration was more wide spread in the group with reflux of pancreatic juice alone, than in the other groups.
Taking all the lesions together, i.e. the erosions, the ulcerations and the hyperkeratosis in the whole oesophagus, these were more extensive in the group with pancreatic juice alone, than in the other groups in which bile and/or gastric juice was reflucting as well.
In the distal part no significant difference in the lesions was found between all groups.
From these findings one might conclude, that when reflux of pancreatic juice either alone or in combination with bile and/or gastric juice was reflucting into the oesophagus, the destructive effect was maximum and equal in the distal part in all experimental groups. If there was an inhibitory effect of bile or gastric juice, then it was not evident in this distal part of the oesophagus.
A distinct inhibitory effect on the occurance and extent of the lesions was however found in the middle part of the oesophagus, also obvious when all the lesions in the whole oesophagus were compared. The destructive effect of the reflucting juices containing pancreatic juice decreased namely in the middle part of the oesophagus, but even more so in the groups where a combination of pancreatic juice with bile and/or gastric juice was reflucting. Simple dilution rather than a specific counteractive effect by bile or gastric juice could have produced this. This was not proven.
In the proximal part of the oesophagus practically no lesions were found in any group. The reflucting juices probably did not reach this part of the oesophagus.

CHAPTER VI

# MICROSCOPIC FINDINGS

## 1. Definitions.

As already mentioned in chapter III. 8 at least two sections of the whole length of the oesophagus were cut from representative locations, for histological examinations.
In this study the histology of a *normal oesophagus* is defined as (see fig. 24): squamous epithelium with a basal layer consisting of one or two rows of cells; a lamina propria consisting of loose connective tissue without signs of edema, a muscularis mucosa, a submucosa and a inner and outer layer of striated

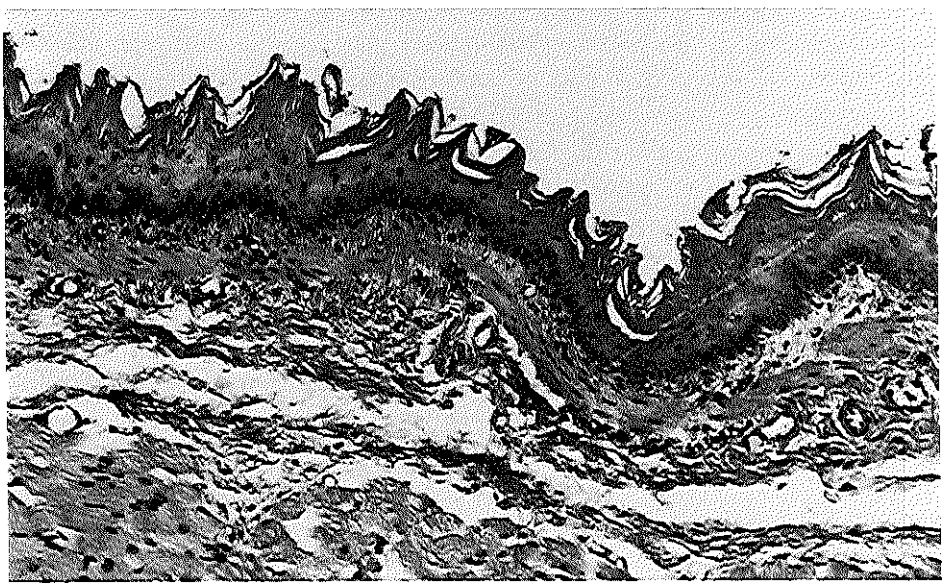


Fig. 24. A part of a normal oesophageal wall, consisting of: squamous epithelium, lamina propria, muscularis mucosa, submucosa and a part of the inner muscle layer H.A.S. x 150.

muscle, surrounded by peri-oesophageal connective and fatty tissue, without any signs of inflammation.
*Erosive lesions* are defined in this study as: a complete or partial destruction of the oesophageal epithelium, without injury to the lamina propria and deeper layers. *Ulcerative lesions* are defined as: a total destruction of the oesophageal epithelium and injury to the lamina propria and possibly the deeper layers of the oesophagus. *Hyperplasia* of the oesophageal epithelium was found and defined as: an increased thickness of the epithelium as a result of cells multiplication, which results in more cells per layer of the epithelium ($>$ 0.038 mm). *Atrofie* of the oesophageal epithelium is defined as: a decrease in the thickness of the epithelium caused by a decrease of cell volume. *Hyperkeratosis* of the oesophageal epithelium is defined as: a hornly layer which is thicker than normal ($>$ 0.009 mm).

**2. Histological changes in chronological sequence.**

On microscopic examination of the oesophagus, changes of the oesophageal wall were only observed in cases of reflux of pancreatic juice and combinations with pancreatic juice (groups G + P + B; G + P; P + B and P). When bile, gastric juice or a combination of these was refluxting, no histological changes were seen in the oesophagus. As there were no clear differences found between the histological changes of the oeosphagi of the individual rats in the same experimental groups, and as there were no significant differences in lesions of the G + P + B; G + P; P + B and P groups all together, the microscopical findings will be described in general, according to the time after the instalment of reflux. As such this gives only a qualitative picture of the oesophagitis which developed. This in contrast to the macroscopic scoring (see chapter III. 7), whereby an impression of the destruction of the whole oesophageal epithelium was obtained.

*3rd day:*

After three days reflux of pancreatic juice or combinations with pancreatic juice, primarily erosive lesions were found in the distal and middle part of the oesophagus.
The lesions were superficial when only part of the epithelium was destructed (see fig. 25), or deep when the whole epithelium was destroyed. In the erosive lesions the lamina propria always remained intact. There was vasodilatation in the submucosa and in the lamina propria. The submucosa and the innermost muscle layer showed an extensive inflammatory infiltrate. This infiltrate consisted primarily of polymorph nuclear leucocytes (P.N.L.) and a

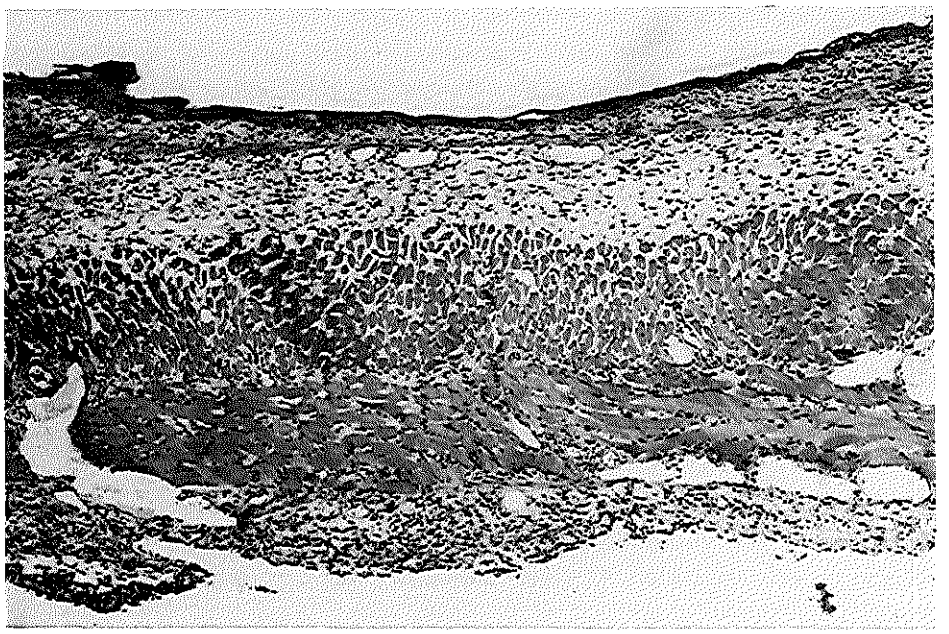


Fig. 25. Erosive lesion, only a part of the epithelium is destructed. Edema and a dense inflammatory infiltrate in the lamina propria and submucosa is visible. H.A.S. x 60.

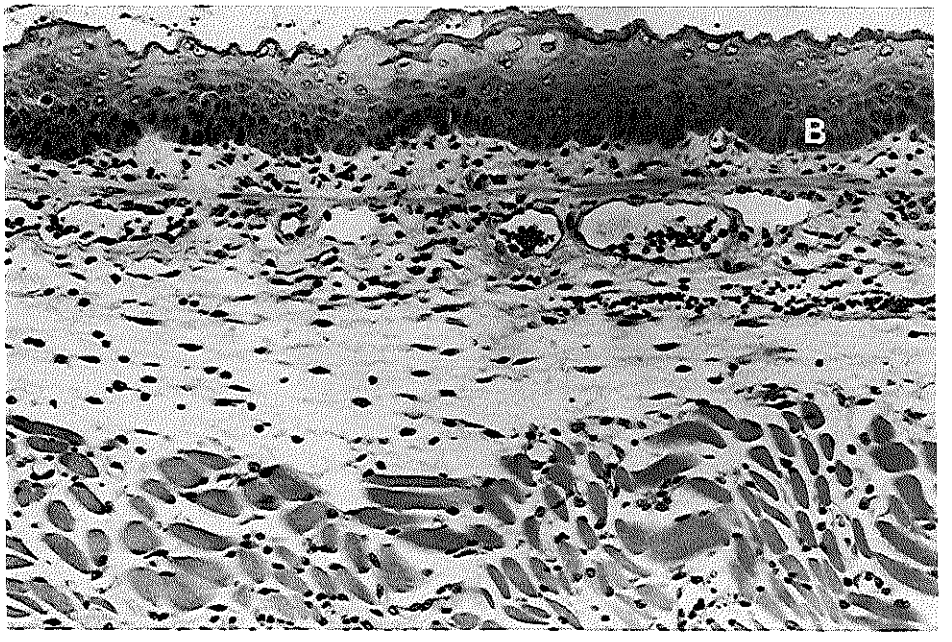



Fig. 26. Increase in basaloid cells. H.A.S. x 150.

few eosinofilic granulocytes. In the erosive parts, there were often punctuate ulcerations with a fibrin covering and fibrinoid necrosis to be found. Underneath these very small ulcerative lesions, there was a more extensive inflammatory infiltrate than in the eroded parts. Around erosive lesions, macroscopical normal mucosa showed on histological examination a transitional area in which the epithelium showed atrofie and there was less inflammatory infiltrate present in the lamina propria beneath. In these same areas also patches of increased mitosis of the basal cell layer and increased basaloid cells (see fig. 26) were found. In the proximal part of the oesophagus, practically no lesions were found at all.

*5th day.*

After 5 days, erosive and ulcerating lesions were found in the distal and middle part of the oesophagus. The edema in the lamina propria and submucosa, was less accentuated than after three days. The inflammatory infiltrate was extended beyond the oesophageal wall into the peri-oesophageal connective and fatty tissue. The lamina propria and the submucosa also showed fibroblasts proliferation.

*7th day.*

After 7 days, mostly ulcerating lesions were seen (see fig. 27) and less erosions. A dense inflammatory infiltrate with round cells and polymorph nuclear leucocytes, penetrated all layers of the oesophageal wall. Eosinofilic degeneration and atrofie of the muscle fibers was detectable together with an increase in the number of fibroblasts and collagen fibres in the lamina propria and submucosa.

*14th day.*

Only ulcerative lesions were seen. There was a dense inflammatory infiltrate in the oesophageal wall and in the peri-oesophageal connective and fatty tissue. In contrast to the findings at earlier stages, the infiltrate consisted chiefly of round cells. The remainder of the epithelium between and around the ulcerative lesions showed increased mitosis and hyperplasia of the basal layer and stratum spinosum (acanthosis), as well as hyperkeratosis. The tissue underneath the hyperplastic epithelium demonstrated only small abnormalities, without fibrosis. Underneath the ulcerative lesions, there was an increase in the amount of collagen connective tissue in the lamina propria and submucosa. Increased atrofie and eosinophilic degeneration of the muscle fibres were also observed.

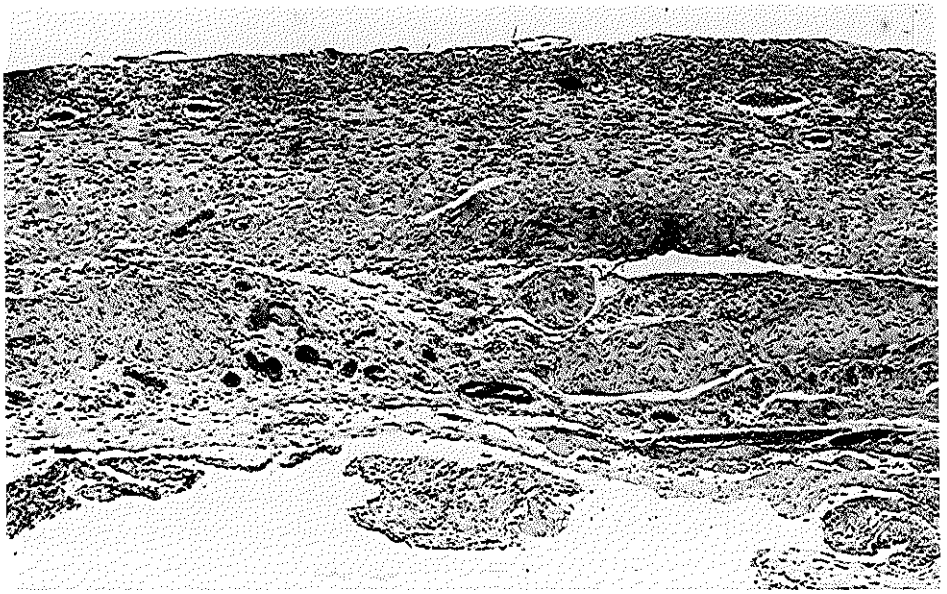


Fig. 27. Ulcerative lesion, the whole epithelium is destructed. A dense inflammatory infiltrate with round cells and polymorph nuclear leucocytes penetrated all layers of the oesophageal wall. H.A.S. x 60.

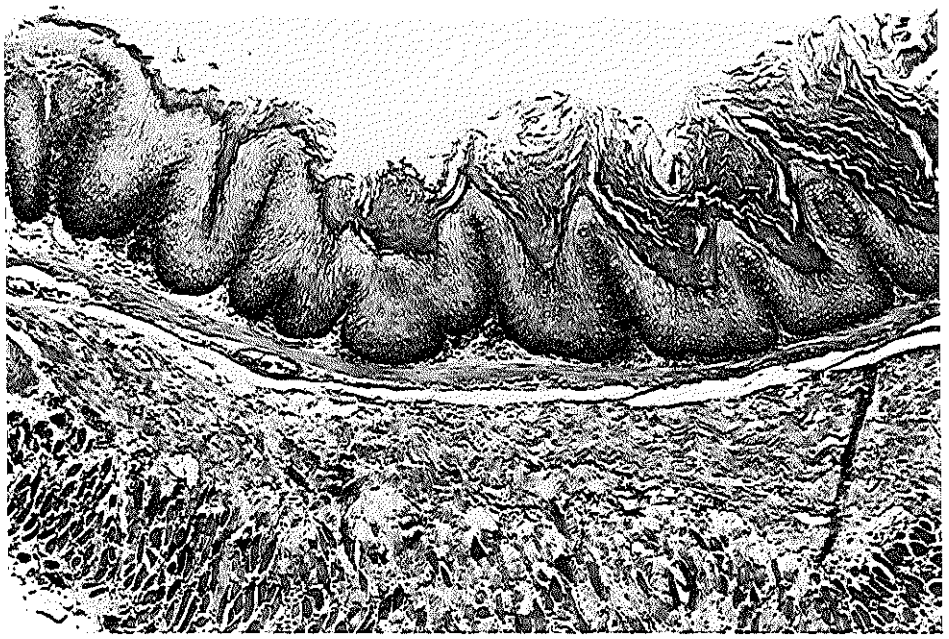


Fig. 28. Oesophageal epithelium with hyperplasia and hyperkeratosis. H.A.S. x 150.

*28th day.*

At this time extensive ulcerations were seen with a dense round cells infiltrate throughout the whole oesophageal wall, as well as in the peri-oesophageal connective and fatty tissue. The remainder of the epithelium between and around the ulcerative lesions demonstrated increased hyperplasia together with increased keratosis (see fig. 28). There was an increase in the collagen connective tissue in the lamina propria, the submucosa and the muscle layers. Increased atrofie and eosinophilic degeneration of the muscle fibres were also present.

*42th day.*

At this stage a further increase in the fibrosis was observed. In some parts of the oesophageal wall, the pre-existing structures were completely replaced by collagen connective tissue (see fig. 29). There was also increased hyperplasia and keratosis of the remaining epithelium between and around the ulcerative areas. Underneath the ulcerative lesions, there was a very dense infiltration with round cells, throughout the whole oesophageal wall visible.

*Metaplasia and a-typical cell formation.*

No metaplasia of the oesophageal epithelium and no a-typical cell formation were found in the sections examined.

**3. Summary of histological findings.**

Table 32 gives a qualitative representation of the findings described with special reference to the time the lesions appeared. And in summary one can say that after three days, mainly erosive lesions and punctuate ulcerations are found in the middle and distal part of the oesophagus. The ulcerations mostly slowly progress in size, while erosions gradually disappear and at 14 days no erosions were found anymore. Hyperplasia is already visible after 3 days in between erosions and ulcerations and is then always found. After 14 days hyperkeratosis become visible. This hyperkeratosis progresses in course of the experiment. Fibrosis can be detected already after 7 days, this is steadily progressive and finally found in the whole oesophageal wall (day 42).

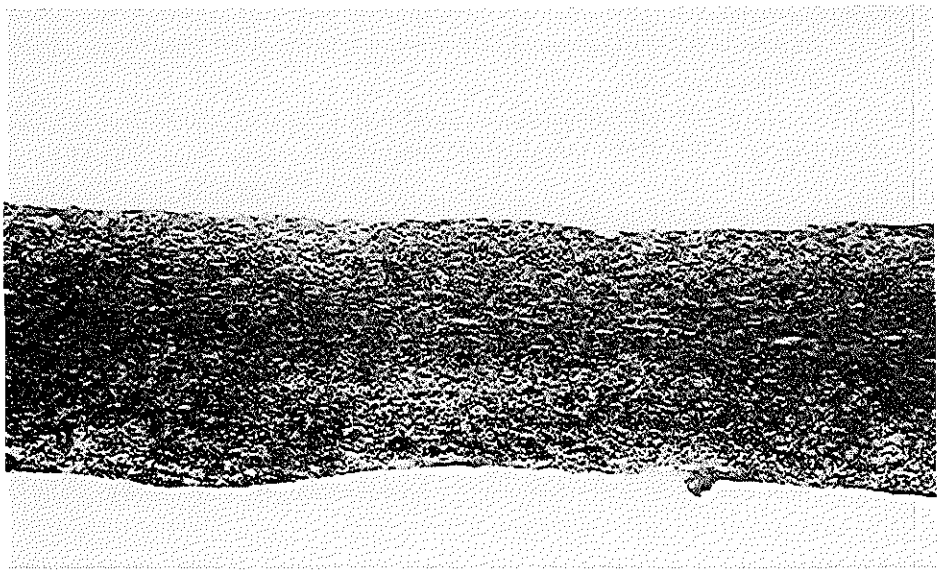


Fig. 29. Ulcerative lesion, the whole epithelium is destructed. Fibrosis of the whole oesophageal wall, with a dense infiltration with round cells throughout the whole oesophageal wall, and loss of pre-existing structures is visible. H.A.S. x 60.

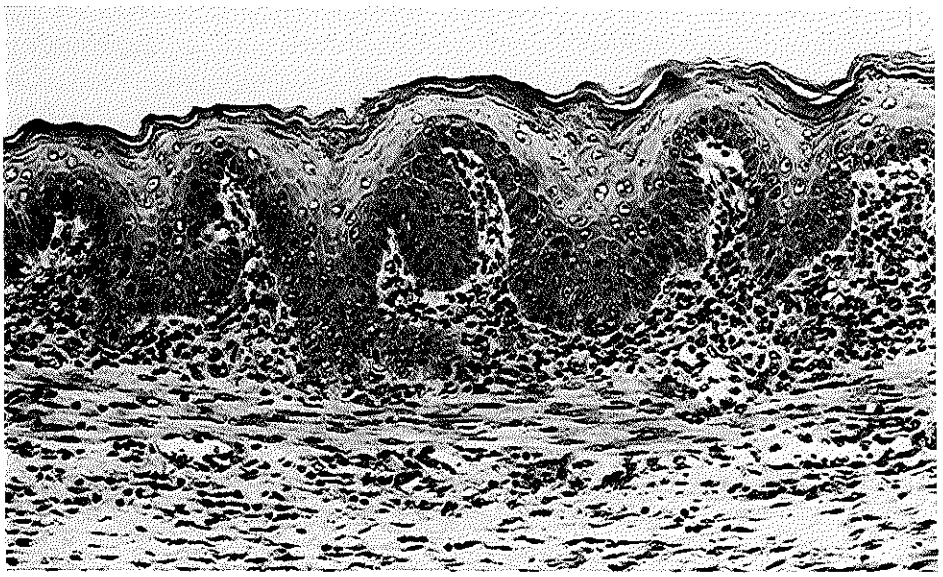


Fig. 30. Transitional area between erosive and normal oesophageal mucous membrane. The epithelium consists of a broad layer of basaloid cells and a thickening of the stratum spinosum (acanthosis), an increase of papillary height and a dense round cells infiltrate in the lamina propria is visible. H.A.S. x 150.

Table 32. Schematic representation of the histological findings in reflux oesophagitis, in the presence of reflux of pancreatic juice or a combination of pancreatic juice with bile and/or gastric juice.

| Time (days) | Erosion | Ulceration | Hyperplasia | Hyper-keratosis | Fibrosis |
|---|---|---|---|---|---|
| 3 | + | ± | + | — | — |
| 5 | + | ± | + | — | — |
| 7 | ± | + | + | — | + |
| 14 | — | + | + | + | + |
| 28 | — | + | + | + | + |
| 42 | — | + | + | + | + |

## 4. Discussion.

The results of the histological examinations of the oesophageal wall, suggests that the lesions which appear, depend on the intensity of the "irritating" stimulus. This might be a matter of concentration of the damaging factor(s) and/or the time of the "irritating" factor(s) is (are) present in the oesophagus. In the presence of reflux of pancreatic juice or combinations containing pancreatic juice, erosive lesions arise initially (3 days). The erosive oesophageal lesions seem to progress slowly into ulcerations in the presence of continued strong "irritation" (day 7). It seems that when the "irritation" is not strong enough to cause erosions and ulcerations, the basal cell layer is stimulated and can react with increased mitosis, so that tickening of the basal layers of the epithelium occurs. This can be found with a fully intact horny layer, or with a slowly eroding horny layer. It seems that the balance between cell destruction and cell formation "divines" the appearance of the total aspect of the epithelium. If the cell formation exceeds the cell destruction, there will be an increase in the thickness of the epithelium layer. This is most clearly visible in the transitional area between erosive and normal oesophageal mucous membrane. Here the epithelium consists of a broad layer of basaloid cells and a thickening of the stratum spinosum (acanthosis) see fig. 30. An increase in the height of the papillae also occurs. The hyperplasia increases in time, which might even eventually result in a thicker keratin layer i.e. hyperkeratosis. After 5 days fibroblastic proliferation in the lamina propria and the submucosa is visible and precipitation of collagen material occurs, which leads eventually to the forming of collagen connective

tissue. This is initially localised in the lamina propria and submucosa (14 days) and can eventually invade the whole oesophageal wall (day 42) see fig. 29).

So from our histology studies a picture of the sequence of the lesions due to pancreatic juice on the oesophageal wall seem to appear (see fig. 31).

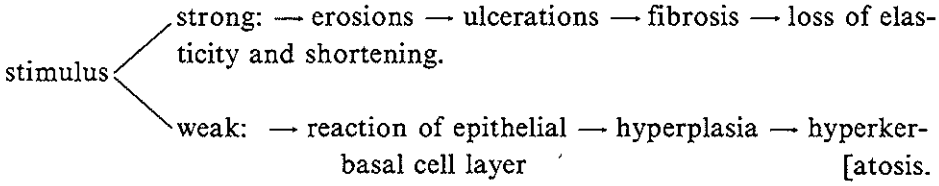



Fig. 31. Sequence of the oesophageal lesions.

As long as the irritating stimulus is weak, only the epithelium is affected. Which obviously might appear on macroscopic examination to be normal. Only when a chronic strong stimulus is present inducing erosions and ulcerations, the process of fibrosis of the oesophageal wall starts, which in the long run might lead to shortening and loss of elasticity of the oesophageal wall. In our studies stenosis was not found. One could speculate that this takes place only on a later stage.

CHAPTER VII

# DISCUSSION AND CONCLUSIONS

In view of the conflicting opinions in the literature on the pathogenesis of reflux oesophagitis in both clinical and experimental studies, an experiment in the rat was performed to analyse which factor(s) in the reflucting juice could be regarded responsible, or partially responsible for the development of reflux oesophagitis. The rats were operated upon in such a way that reflux of pancreatic juice, bile and gastric juice or their combinations could be studied. In this study the term pancreatic juice is used for simplicity and refers to duodenal juice without bile. Gastric juice and bile both have been proposed as etiological factors (Casten, 1967, Mackman et al, 1971 and Burge and Amery, 1973), but the importance of pancreatic juice per se has never been stressed in this context. The method used in our study to score the oesophageal lesions, showed that lesions only occurred in the presence of reflux of pancreatic juice or its combination with bile and/or gastric juice. Addition of bile or gastric juice seemed to produce an inhibitory rather than potentiating effect. From our studies it was not definite clear if this was due to dilution or if bile and/or gastric juice actually counteracted the destructive effect of pancreatic juice. Furthermore one can critize that duodenal juice with pancreatic juice has been tested instead of pancreatic juice alone. However, Levrat et al (1962) showed that duodenal juice without bile and pancreatic juice had no destructive effect on oesophageal mucosa in a comparable rat experiment. A group of rats in which an oesophago-jejunostomy and a distal jejuno-jejunostomy according to Roux en Y was performed, did not develop oesophagitis. Our results agree with those of Levrat et al (1962). These workers did not demonstrate the presence of reflux radiographically. In contrast to our results, a potentiation of the destructive action of pancreatic juice by the addition of bile was found.

As far as we know, in the literature dealing with human reflux oesophagitis, no attention has been paid to pancreatic juice as a possible factor in the genesis of human reflux oesophagitis so far. Holt and Large (1961) described a number of patients whereby oesophagitis occured after a cardioplasty for

cardiospasm, which persisted in spite of a subtotal gastric resection to reduce the acid production. Analysis of the secretion in the oesophagus of these patients showed high content of bile and amylase. The authors concluded that factors in the duodenal juice were responsible for the persisting oesophagitis, however, without specifying which factor(s) could be responsible.

Many animal experiments were carried out to test the theory that bile was an etiological factor. These experiments were in the main unphysiological "drip" tests. Different concentrations of bile or bile salts were used, and duration of the local "perfusion" varied orderly. Moffat and Berkas (1965) found in the dog that lesions could be caused by "perfusion" with bile as well as with bile salts. Gillison et al. (1972), performed anastomosis of the gallbladder with stomach with excision of the cardia in the monkey. This resulted in serious ulcerative lesions in the oesophagus. Gillison concluded that bile contaminated with gastric juice was responsible for the development of reflux oesophagitis. He did not take account to exclude the reflux of pancreatic juice into the stomach as a result of pyloric insufficiency. The clinical studies of Scott and Weidner (1956), Balint and Gummer (1958) and Gillison et al (1972), demonstrate that an oesophagitis proceeding a total gastric resection was preventable by an oesophago-jejunostomy and a distal jejuno-jejunostomy according to Roux en Y. The authors postulated that these studies indicated that bile was the major factor in the development of reflux oesophagitis (although pancreatic juice is deviated as well). Stol and Collis (1974) and Crumplin et al (1974) demonstrated that patients with a "symptomatic" hiatal hernia have an increased total bile acid concentration in their gastric juice. This indicated an insufficiency of the pylorus in these patients. The authors concluded that the degree of the pathological response in the oesophagus appeared to correlate with an increased total bile acid concentration. Our experiment shows that the reflux of the bile alone causes no lesions in the oesophagus. Likewise reflux of bile together with gastric juice caused no visible lesions. In two groups, where gastric juice or gastric juice together with bile was refluсting into the oesophagus, reflux of duodenal contents into the stomach was theoretically possible. However, when the results of these groups were compared with the groups in which reflux of pancreatic juice into the oesophagus occurred, it was concluded that if duodeno-gastric reflux did occur, this could not be of such a degree that it interfered with our final interpretations.

There is much controversy over the role of gastric juice (i.e. gastric acid and pepsin) in the etiology of oesphagitis both in clinical and laboratory studies.

Seleye (1938) and Lodge (1954) found that reflux of gastric juice in rats could cause oesophagitis. In contrast to Lambert (1962) and Levrat et al. (1962), who found no oesophagitis when reflux of gastric juice occured. Results similar to the latter were obtained in our experiment. Dog experiments suggest that gastric juice does play a role in development of reflux-oesophagitis. However, evidence is limited mainly to "drip" experiments of variable duration and concentrations of acid and pepsin (Kiriluk and Merandino, 1954, Redo et al, 1959, Barnes et al, 1968). The hypothesis that gastric juice could be significant in the development of reflux oesophagitis in man, seemed further to be supported by the fact that oesophagitis occurs often in combination with an increased acid secretion in the stomach (Stol and Collis, 1974). Further Casten et al (1963) and Burge et al (1966) showed that duodenal ulcerations were frequently associated with oesophagitis.
Arguments against the gastric juice reflux theory, however, are: the fact that oesophagitis occurs in non-acid producing patients (Palmer 1960) and that after total gastric resection, patients can also develop an oesophagitis (Helsingen 1960). The fact that an agent such as cimetidine, a powerful acid-pepsin inhibitor, does not always cure an oesophagitis is an argument against the acid-pepsin theory (Powel Jackson et al 1978, Behar et al 1978, Ferguson et al 1979). It is of note however, that the symptoms such as retrosternal burning, usually disappear with cimetidine therapy. This could indicate that there is less reflux of acid into the oesophagus, so relieving the symptoms, however oesophagitis is still present.

Our study proves that lesions of the oesophagus only occur with reflux of duodenal juice containing pancreatic juice alone or in combination with bile and/or gastric juice. The data suggests strongly that reflux of pancreatic proteolytic enzymes could be the main factor inducing oesophagitis. Recently Mud et al (1980) demonstrated that in comparable experimental circumstances, trypsin could be detected in the oesophagus of rats with reflux of pancreatic juice and combinations of pancreatic juice with bile and/or gastric juice.
It is assumed that human reflux oesophagitis can only occur when proteolytic pancreatic juice refluxes into the oesophagus, then cardio-oesophageal insufficiency must be shown to be accompanied by a pyloric insufficiency. As mentioned Stol and Collis (1974) and Crumplin et al (1974) demonstrated an increased pyloric reflux of bile acids in patients with reflux-oesophagitis. Capper et al (1966) demonstrated pyloric reflux in patients with cardio-oesophageal insufficiency, by means of radiological contrast medium introduced into the duodenum. If pyloric insufficiency with reflux of bile and

radiological contrast into the stomach exists, reflux of pancreatic juice will also occur. Wenger and Trowbridge (1971) showed in patients with an ulcus ventriculi or duodeni that reflux of duodenum contents into the stomach after a test meal occurs regularly, and that trypsin is detectable in the stomach.
In a pilot study our group analysed gastric juice of symptomatic and asymptomatic patients with gastro-oesophageal reflux. Trypsin was found in all patients tested, irrespective symptoms or not. Most interesting however, was that the concentration of trypsin in the gastric juice was significantly higher in patients with symptoms or signs of oesophagitis than in patients without, or control patients. Further clinical studies to verify these findings, and analysis of the content of the oesophagi in patients with reflux oesophagitis are needed.

The histological examination in this thesis showed that when reflux of pancreatic juice or combinations with pancreatic juice were induced, extensive erosive destruction of the oesophageal mucous membrane occured within 3 days. The erosive oesophageal lesions change slowly to ulcerations in the presence of continued "irritant" stimulus. Fibrosis was obvious after 7 days. This fibrosis appeared in almost the whole oesophageal wall in the course of time and resulted in shortening and loss of elasticity. Stenosis was not found during the observation period (42 days). In some parts of the oesophagus with macroscopically normal mucosa, there was nevertheless stimulation or the basal cell layer demonstrated by an increase in mitotic activity and cell proliferation. This phenomena was most evident in the transitional area between erosive and/or ulcerative lesions and normal oesophageal mucous membrane. Further maintenance of the stimulus seemed to cause acanthosis and increased papillary height in these areas. The inflammatory infiltrate in the lamina propria here was slight. This hyperplasia of the oesophageal mucosa in the rats is most probably caused by a chronic irritation of pancreatic juice, which however in these areas is not so strong a stimulus as to evoke erosions and ulcerations. These histological findings in our rat studies resemble the histology of human oesophagitis (Ismail-Beigi et al, 1970) found in the presence of macroscopically normal mucosa. It was suggested that in man also this histological pattern in due to a chronic moderate irritating stimulus in the refluctingjuice. With reference to the generally propagated view that chronic "irritation" of the oesophageal mucosa could induce malignant cell transformation, our histological study showed that inspite of increased mitotic activity and cell proliferation, atypical cell formation and/or metaplasia could not be found

In the literature on human oesophagitis the term peptic and biliary reflux oesophagitis are used extensively at present. From the results of our study and of the literature mentioned, it is proposed to avoid these terms. For the human situation the terms gastro-oesophageal reflux and reflux-oesophagitis should be used until on a later date when the precise etiological factor(s) of oesophagitis is (are) known.

Reviewing the objectives of the thesis (chapter I) the following conclusions can be drawn:

1. Definite lesions of the oesophageal wall only occur with reflux of pancreatic juice or its combination with bile and/or gastric juice. These lesions consisted macroscopically of erosions, ulcerations and hyperkeratosis.
2. Macroscopical examination of the oesophageal mucosa showed erosive lesions already in the initial stage (3 days) after initiating reflux. The erosive lesions changed slowly to ulcerations in the presence of continued "irritation". Hyperkeratosis was also found, it was first observed on day 14 and then increased in time.
3. In the presence of reflux of pancreatic juice or combinations of it, the distal part of the oesophagus was always more subject to destruction than the middle and proximal parts. The proximal part of the oesophagus showed almost no lesions.
4. Some parts of the oesophageal mucous membrane appeared normal on macroscopic examination (especially the trasitional area between erosive and/or ulcerative and normal oesophageal mucous membrane). However, these were found histologically abnormal e.g. hyperplasia of the basal layer, acanthosis, increase in papillary height, often combined with a slight inflammatory infiltrate in the lamina propria being present. This hyperplasia, probably induced by mild chronic irritation, seemed the basis for the hyperkeratosis which developed on a later stage.
5. Oesophageal tissues subject to the influence of pancreatic juice showed definite microscopic evidence of an increase in connective tissue, within 7 days. This connective tissue formation became more extensive through the course of the experiment. No stenosis developed within 42 days of follow-up in our experiment.
6. After 42 days of pancreatic juice reflux a loss of elasticity and shortening of the oesophagus was observed. This was most probably due to the progressive extensive fibrosis throughout the layers of the oesophageal wall.
7. In the histological slides examinated, no atypical cell formation and/or metaplasia was observed.

8. Oesophago-jejunostomy and distal jejuno-jejunostomy according to Roux en Y without gastric resection, effectively prevented oesophagitis in our experimental model.

## SUMMARY

The objectives of this thesis were to analyse the influence of gastric juice, bile and pancreatic juice on the development of reflux oesophagitis on one hand, and on the other hand to get more information on the kinetics of developing oesophagitis.
The motivation for this experimental study on rats is given in *chapter I.*

*Chapter II* contains a survey of the literature on factors which have been suggested to be involved in the etiology of reflux oesophagitis in man as well as in experimental animal studies. The oesophago-jejunostomy and distal jejuno-jejunostomy according to Roux en Y as operation to treat or to prevent reflux oesophagitis is discussed.
The correlation between the macroscopic and microscopic pictures of reflux oesophagitis in men are described.
Details of the laboratory animals, experimental groups, operative techniques and post-operative analysis, and further details about the experimental set-up are given in *chapter III.* This chapter explains the scoring system for quantifying the macroscopically visible oesophageal lesions, and describes the histological techniques and the technique of radiographic examination.

*Chapter IV* contains the general results of the studies. The operation times, the size of the experimental groups, the complications and the changes in body weight after operation is given. There were nine different experimental groups. Reflux of gastric juice (G) together with bile (B) and pancreatic juice (P) was obtained by performing an oesophago-jejunostomy. By adding a deviation procedure of the choledochus duct and/or a total gastric resection, different experimental groups with different types of reflux could be formed. Two control groups consisted of rats with a laparotomy alone (sham group) and a group consisting of rats with an oesophago-jejunostomy and a jejuno-jejunostomy 25 cm distal from the former anastomosis (Roux en Y group). From the weight curves it was apparent that rats which did develop oesophagitis lost weight and continued to do so.

The technique used for biliary deviation seemed to be succesfull in 95.7% of the cases, as demonstrated by serum bilirubin estimations.
Radiographic examination of two rats from each of the experimental groups except the Roux en Y and the Sham group, demonstrated oesophageal reflux of contrast medium via the oesophago-jejunostomy into the oesophagus. In none of the five normal control rats, reflux of contrast was seen.
Autopsy demonstrated eight deaths (of a total of 195 rats operated upon) to be due to peritonitis. Six of these expired from perforation of an oesophago-jejunostomy, and two from dehiscence of an implanted bile duct. All intestinal anastomoses remained in tact. Perforation of the oesophagus did not occur. In 5 rats passage of a silastic catheter (∅ 4 mm) through the oesophago-jejunostomy was not possible. Faulty technique or granuloma formation due to stich material is thought to have produced this stenosis.
On macroscopical examination of the oesophagus only in the group with reflux of pancreatic juice (P) or pancreatic juice and/or bile (B) and gastric juice (G) were erosions, ulcers and hyperkeratosis found. In all other groups no lesions were found.
Loss of elasticity of the oesophageal wall had occured by the 28th day in groups G + P + B, P + B, G + P and P. A further reduction in elasticity had occured by the 42nd day. These groups also showed shortening of the oesophagus; by day 28 of 0.5 - 0.75 cm and by the 42nd day of 0.75 - 1 cm (total length of normal oesophagus 7 - 8 cm).

*Chapter V* gives the results of the analysis of the lesions found in the oesophagus. Using a scoring system a statistically significant overal-difference between the group with pancreatic reflux alone, and the group in which pancreatic reflux with bile and/or gastric juice was demonstrated. Bile and/or gastric juice in combination with pancreatic juice, seemed to diminish the "destructive" effect of pancreatic juice.

In *chapter VI* the microscopic findings are given. The histological studies described is this thesis showed that when reflux of pancreatic juice or combination with pancreatic juice were initiated, extensive erosive destruction of the oesophageal mucous membrane occured already after 3 days. The erosive lesions changed slowly to ulcerations in the presence of continued "irritating" stimulus. In some parts of the oesophagus with macroscopic normal mucosa, there was nevertheless stimulation of the basal cell layer, demonstrated by an increase in mitotic activity and cell proliferation. This phenomena was most evident in the transitional area between erosive and/or ulcerative lesions and normal oesophageal mucous membrane. Maintenance of the stimulus further seemed to cause acanthosis

and increased papillary height in these areas. The hyperplasia of the oesophageal mucosa in the rat is most probably caused by a chronic influence of pancreatic juice, which is however in these areas not so strong a stimulus to evoke erosions and ulcerations. An obvious beginning fibrosis was visible after 7 days. This fibrosis invaded almost the whole oesophageal wall in the course of time, and resulted in shortening and loss of elasticity (42 days).

In *chapter VII* the discussion and the conclusions from our studies are given.

## SAMENVATTING

Het doel van het onderzoek in dit proefschrift beschreven was om enerzijds de invloed van maagsap, gal en pancreassap op de ontwikkeling van de reflux oesophagitis te bestuderen en anderzijds om meer inzicht te verkrijgen in de kinetiek van de zich ontwikkelende oesophagitis

In *hoofdstuk I* worden de concrete vragen die de basis van deze studie vormden beschreven.

In *hoofdstuk II* wordt een literatuur overzicht gegeven van de mogelijke oorzakelijke factoren die betrokken kunnen zijn bij de ontwikkeling van de reflux oesophagitis bij de mens. Een kort overzicht van de verrichtte dierexperimenten wordt gegeven. De oesophago-jejunostomy volgens Roux en Y ter behandeling en voorkoming van een reflux oesophagitis wordt eveneens besproken. Vervolgens wordt ingegaan op de relatie tussen macroscopische en microscopische bevindingen bij patienten met een reflux oesophagitis.

In *hoofdstuk III* worden de proefdieren, de experimentele groepen, de operatie technieken, de post-operatieve zorg en de verdere details over de experimentele opzet beschreven. In dit hoofdstuk wordt eveneens het gebruikte score systeem ter kwantificering van de slokdarm laesies, de histologische technieken en het uitgevoerde röntgenonderzoek besproken.

In *hoofdstuk IV* worden de resultaten vermeld. De operatietijden, de grootte van de experimentele groepen, de complicaties en het gewichtsverloop worden besproken. Er waren 9 verschillende experimentele groepen. Reflux van maagsap (G) tezamen met gal (B) en pancreassap (P) werd verkregen door middel van een oesophago-jejunostomie. Wanneer hierbij wel of geen totale maagresectie en/of ductus choledochus deviatie werd uitgevoerd, kon reflux bewerkstelligd worden van maagsap (G), gal (B) of pancreassap (P) afzonderlijk. Twee groepen waren controle groepen, deze bestonden uit ratten waarbij alleen een laparotomie werd uitgevoerd (Sham groep) en een groep

waarbij een oesophago-jejunostomie en een jejuno-jejunostomie 25 cm distaal van de eerste anastomose werd uitgevoerd (Roux en Y groep).
Uit de gewichtscurves bleek dat ratten die een oesophagitis ontwikkelden voortdurend in gewicht afnamen.
De gebruikte methode van ductus choledochus deviatie bleek in 95,7% succesvol te zijn, zoals bleek uit de serum bilirubine bepalingen.
Röntgenonderzoek bij steeds twee ratten uit alle experimentele groepen toonde aan dat bij alle ratten, behalve die van de Roux en Y en Sham-groep, contrast via de oesophago-jejunostomie in de slokdarm reflucteerde. Bij 5 normale controle ratten bleek er nooit contrast in de slokdarm te reflucteren.
Bij obductie bleken 8 van de in totaal 195 geopereerde ratten te zijn overleden t.g.v. een peritonitis. Dit was zesmaal het gevolg van perforatie van de oesophago-jejunostomie en tweemaal t.g.v. dehiscentie van de gereïnplanteerde ductus choledochus. Alle darmanastomosen bleken sufficient. Er werden geen slokdarmperforaties gevonden.
Bij 5 ratten bleek een silastic canule met een doorsnede van 4 mm de oesophago-jejunostomie niet te kunnen passeren, mogelijk was een fout in de anastomose techniek of granuloomvorming t.g.v. het gebruikte hechtmateriaal verantwoordelijk voor deze stenose.
Alleen na reflux van pancreassap of combinaties van pancreassap met gal en/of maagsap ontstonden slokdarm laesies. Deze laesies bestonden uit: erosies, ulceraties en op den duur hyperkeratotische verschijnselen. In al de andere groepen werden geen laesies gevonden.
Na 28 dagen bleek er bij de groepen G + P + B, P + B, G + P en P elasticiteitsverlies van de slokdarm te zijn opgetreden. Dit was na 42 dagen nog verder toegenomen. Bij bovengenoemde groepen bleek de slokdarm na 28 dagen 0.5 - 0.75 cm te zijn verkort en na 42 dagen 0.75 - 1 cm (totale lengte van een normale slokdarm 7 - 8 cm).

*Hoofdstuk V* bevat de resultaten van statistische analyse van de gevonden slokdarm laesies. Door gebruik te maken van een score systeem bleek er een statistisch significant overal-difference te bestaan tussen de groep waarbij alleen pancreassap reflux was bewerkstelligd en de groepen waarbij pancreassap tezamen met gal en/of maagsap had gereflucteerd. Gal en/of maagsap in combinatie met pancreassap leek een remming van het "destructieve" effect van pancreassap te bewerkstelligen.

In *hoofdstuk VI* worden de microscopische bevindingen beschreven. Na reflux van pancreassap of een combinatie van pancreassap met gal en/of maagsap ontstonden reeds in een vroeg stadium (3 dagen na de operatie) uitgebreide erosieve laesies van het slokdarm slijmvlies. De erosieve laesies

gingen bij aanhouden van de laederende "prikkel" langzamerhand over in ulceraties.
In sommige gedeelten van de slokdarm waarbij het slijmvlies er macroscopisch normaal uitzag, bleek de basale cellaag echter geprikkeld te zijn, hetgeen zich uitte in toegenomen mitose activiteit en celproliferatie. Dit fenomeen was het duidelijkst zichtbaar in het overgangsgebied tussen erosieve en/of ulceratieve laesies en normaal slokdarm slijmvlies. Continuering van de stimulus leek in deze gebieden verder aanleiding te geven tot acanthose en toename in papil hoogte. Deze hyperplasie van het slokdarmslijmvlies van de rat wordt zeer waarschijnlijk veroorzaakt door een chronische invloed van pancreassap. Deze prikkel is echter in deze gebieden vermoedelijk niet zo sterk dat dit aanleiding geeft tot het ontstaan van erosies en ulceraties.
Na 7 dagen was er een duidelijke beginnende fibrosering van de slokdarmwand zichtbaar. Deze fibrosering nam in de loop van de tijd praktisch de gehele slokdarmwand in beslag en gaf aanleiding tot verkorting en elasticiteitsverlies (42 dagen).

*Hoofdstuk VII* bevat een discussie over de verkregen resultaten van het onderzoek. Deze discussie wordt afgesloten met een korte opsomming van de conclusies die uit het onderzoek getrokken konden worden.

## REFERENCES


ADLER, R.H., LIBASSI, P.J., STOLL, H.C., BUFFALO, N.Y.: Comparative resistance of the oesophagus to acid and pepsin. Surgery. 44 : 795, 1958.

ALLISON, P.R.: Peptic ulcer of the oesophagus. Thorax. 3 : 20, 1948.

ALLISON, P.R.: Reflux-oesophagitis, sliding hiatus hernia, and the anatomy of repair. S.G.O. 92 : 419, 1951.

ALLISON, P.R., JOHNSTONE, A.S.: The oesophagus lined with gastric mucous membrane. Thorax. 8 : 87, 1953.

ATKINSON, M., VAN GELDER, A.: Oesophageal intraluminal pH recording in the assessment of gastro-oesophageal reflux and its consequences. Am. J. of Digestive. Diseases. 22 : 365, 1977.

BALINT, J.A., GUMMER, J.W.P.: Total gastrectomy for benign peptic ulcers: The Lancet, page 1044-1048, 1958.

BARNES, W.A., REDO, S.F., SCHWARTZ, L.: Vulnerability of various levels of the oesophagus to peptic digestion. Amer. J. of Surg. 115 : 276, 1968.

BEHAR, J., SHEEHAN, D.C.: Histologic abnormalitis in reflux-oesophagitis. Arch. Pathol. 99 : 387, july 1975.

BEHAR, J., BRAND, D.L., BROWN, F.C., CASTELL, D.O., et al.: Cimetidine in the treatment of symptomatic gastro-oesophageal reflux. Gastro-enterology. 74 : 441, 1978.

BEHAR, J.: Surgical Treatment of reflux oesophagitis. How well does it work? Gastroenterology vol 77 no. 1 : 183, 1979.

BOEREMA, I., GERMS, R.: Fixation of the lesser curvature of the stomach to the abdominal wall after reposition of the hernia through the oesophageal hiatus. Arch. Chir. Neerl. 7 : 351, 1955.

BOEREMA, I.: Gastropexia anterior geniculata for sliding hiatus hernia and for cardiospasm. J. Internat. Coll. Surg. 29 : 533, 1958.

BOEREMA, I.: Hiatus hernia: repair by right-sided, subhepatic, anterior gastropexy. Surgery. 65 : 884, 1969.

BRAIN, R.: Peptic strictures of the oesophagus associated with duodenal ulcers and operations for its relief. Proc. Roy. Soc. Med. 59 : 929, 1966.

BURGE, H., GILL, M.G., MACLEAN, C.D.T.: Thorax 21 : 66, 1966.

BURGE, H., AMERY, A.H.: Vagotomy for peptic oesophageal stricture. Ann. R. Coll. Surg. Engl. 53 : 189, 1973.

CAPPER, W.M. et al.: A test for pyloric regurgitation lancet. 2 : 621, 1966.

CASTEN, D.F., BERNHANG, A., NACH, R.J., SPINZIA, J.: A physiological basis for the surgical treatment of sliding oesophageal hiatal hernia. S.G.O. 117 : 87-93, 1963.

CASTEN, D.F.: Peptic oesophagitis, hiatus hernia and duodenal ulc. Am. J. of Surg. 113 : 638, 1967.

COLLIS, J.L.: The causes of the variable response to gastric reflux in hiatus hernia. J. Royal. Coll. Surg. Edinb. 15 : 77, 1970.

CROSS, S., WANGENSTEIN, D.H.: Role of bile and pancreatic juice in production of oesophageal erosions and anemia. Proc. Soc. Exp. Biol. and Med. 77 : 862, 1951.

CRUMPLIN, M.K.H., STOL, D.W., MURPHY, G.M., COLLIS, J.L.: The pattern of bile salt reflux and acid secretion in sliding hernia. Brit. J. Surg. 61 : 611, 1974.

DeMEESTER, T.R., JOHNSON, L.F.: "Evaluation of current operations for the prevention of gastro-esophageal reflux". Ann. Surg. 180 : 511, 1974.

DeMEESTER, T.R., JOHNSON, L.F., JOSEPH, G.J., et al.: Patterns of gastro-esophageal reflux in health and disease. Ann. Surg. 184 : 459, 1976.

DUNPHY, J.E., WAY, L.W.: Current surgical diagnosis and treatment. Los Altos, California, 1979.

ELLIS, F.H., HOOD, R.T.: Exp. oesophago-gastrectomy related of type of resection to development of oesophagitis. S.G.O. 98 : 449, 1954.

ELLIS, F.H.: A physiologic operation for ulceration and stricture of the terminal oesophagus. Proc. Staff. Meetings. of the Mayo. Clinic. 31 : 615, 1956.

ELLIS, F.H., LEONARDI, H.K.: Surgery for short oesophagus with stricture. An experimental and clinical manometric study. Annals. of Surg. 188 nr. 3, sept. 1978.

FERGUSON, D.J., SANCHEZ-PALOMERA, E., et al.: Studies on experimental oesophagitis. Surgery. 28 : 1022, 1950.

FERGUSON, R., DRONFIELD, M.W., ATKINSON, M.: Cimetidine in treatment of reflux oesophagitis with peptic stricture. Br. Med. Journal, 2 : 472, 1979.

GILLISON, E.W., CAPPER, W.M., GIBSON, M.J., BRADFORD, I.: Hiatus hernia and heartburn. GUT. 10 : 609, 1969.

GILLISON, E.W.: Bile reflux, gastric secretion and heartburn. Brit. J. Surg. 58 : 864, 1971.

GILLISON, E.W., BOMBECK, C.T., NYHUS, L.M.: The significance of bile in reflux oesophagitis. Brit. J. Surg. 59 : 794, 1972.

GILLISON, E.W., DE CASTRO, M.D., NYHUS, L.M.,KOJI KUSAKARI, F.A.C.S., BOMBECK, C.: The significance of bile in reflux oesophagitis. S.G.O. 134 : 419, 1972.

HELSINGEN, N., Jr.: Oesophagitis following total gastrectomy. Acta. Chir. Scand. 118 : 190, 1960.

HELSINGEN, N., Jr.: Oesophagitis following total gastrectomy in rats in relation to type of reconstruction. Acta. Chir. Scand. 119 : 230, 1960.

HENDERSON, R.D., MUGASHE, F., JEEJEEBOY, K.N., CULLEN, J., SZCZEPANSKI, M., BOSZKO, A.: The role of bile and acid in the production of oesophagitis and the motor defect of oesophagitis. The Annals. of Thoracic. Surg. 14 : 465, 1972.

HENDERSON, R.D., MUGASHE, F.L., JEEJEEBOY, K.N., CULLEN, J., SZCZEPANSKI, M., BOSZKO, A.: Synergism of acid and bile salts in the production of experimental oesophagitis. The Canadian J. of Surg. 16 : 12, 1973.

HILL, L.D.: An effective operation for hiatal hernia: an eight year appraisal. Ann. surg. 166 : 681, 1967.

HOLT, C.J., LARGE, A.M.: Surgical management of reflux oesophagitis. Am. J. of surg. 153 : 555, 1961.

ISMAIL-BEIGI, F., HORTON, P.F., POPE, C.E.: Histological consequences of gastro-esophageal reflux in man. Gastro-enterology. 58 nr. 2 : 163, 1970.

JACKSON, C.: Peptic ulcer of the oesophagus. J.A.M.A. 92 : 369, 1925.

JOHNSON, L.F., DeMEESTER, T.R.: Twenty-four hour pH monitoring of the distal oesophagus. Am. J. gastroenterol. 62 : 325, 1974.

JONHNSON, L.F., DeMEESTER, T.R.: Medical therapy for gastro-esophageal reflux assessed by 24-hour pH monitoring. Gastroenterol 70 : 897, 1976.

KIRILUK, L.B., MERENDINO, K.A.: The comparative sensitivity of the mucosa of the various segments of the alimentary tract in the dog to acid-peptic action. Surgery. 35 : 547, 1954.

KOBAYASHI, S., KASUGUI, T.: Endoscopic and biopsy criteria for the diagnosis of oesophagitis with a fiberoptic oesophagoscope. Digestive. Disease. vol 19 nr. 4 : 345, 1974.

KRANENDONK, S.E., WESTBROEK, D.L., OBERTOP, H., TEN CATE, F.J.W., VAN HOUTEN, H: Experimental reflux oesophagitis in the rat. European surgical research. Vol 11, supplement 2, page 12, 1979.

KRANENDONK, S.E., WESTBROEK, D.L., OBERTOP, H., TEN CATE, F.J.W., VAN HOUTEN, H.: Experimentele reflux oesophagitis bij de rat. Ned. T. v. Geneeskunde, nr. 51, 2206, 1979.

LAMBERT, R.: Surgery of the digestive system in the rat. Charles Thomas, Springfield - Illinois, 1965.

LAMBERT, R.: Relative importance of biliary and pancreatic secretion in the genenis of oesophagitis in rats. Amer. J. of Dig. Diseases. 7 nr. 11, 1962.

LEVRAT, M., LAMBERT, R., KIRSHBAUM, G.: Oesophagitis produced by reflux of duodenal contents in rats. Amer. J. of Dig. Diseases. 7, nr. 6, 1962.

LODGE, K.V.: Experimental oesophagitis in rats. Br. J. of Exp. Pathology. 36 : 155, 1954.

MACKMAN, S., LEMMER, K.E., MORRISEY, J.F.: Postoperative reflux alkaline gastritis and oesophagitis. The Amer. J. of Surg. 121 : 694, 1971.

MOFFAT, R.C., BERKAS, E.M.: Bile oesophagitis. Arch. Surg. 91 : 963, 1965.

MUD, H.J., KRANENDONK, S.E., OPBERTOP, H., WESTBROEK, D.L., VAN HOUTEN, H.: Duodenal reflux oesophagitis in the rat. European surgical research suppl. I no. 42 page 38, 1980.

NISSEN, R., ROSSETTI, M.: Die Behandlung von Hiatus hernien und Reflux oesophagitis mit Gastropexie und Fundoplicatio. Indikation, Technik und Ergebnisse. Stuttgart: Thieme 1959 -: Surgery of Hiatus Hernia. Med. World. 91 : 20, 1959.

NISSEN, R.: Gastropexy and "fundiplication" in surgical treatment of hiatal hernia. Am. J. Dig. dis. 6 : 954, 1961.

ORLANDO, R.C., BOZYMSKI, E.M.: Heartburn in pernicious anemia. A consequence of bile reflux. The New England. J. of Med. 6 : 522, 1973.

PALMER, E.D.: Subacute erosive ("peptic") oesophagitis. A.M.A. Archives ofIntern. Med. 94 : 364, 1954.

PALMER, E.D.: Subacute erosine ("peptic") oesophagitis associated with achlorhydria. New. Eng. J. Med. 262 : 927, 1960.

POWELL-JACKSON, P., BARKLEY, H., NORTHFIELD, T.C.: Effect of cimetidine in symptomatic gastro-oesophageal reflux. Lancet. 2 : 1068, 1978.

QUINCKE, H.: Ulcus oesophagi ex digestione. Deutsches Archiv. für Klin. Medizin, 24 : 72, 1879.

REDO, S.F., BARNES, W.A.: Effects of the secretions of the stomach, duodenum, jejunum and colon on the oesophagus of the dog. S.G.O. 106 : 337, 1958.

REDO, S.F., BARNES, W.A., DE LA SIERRA, A.O.: Perfusion of canine oesophagus with secretion of the upper gastro-intestinal tract. Annals of surg. 149 no. 4 : 556, 1959.

ROSSETTI, M.: Die Reflux Krankheit des Oesophagus. Hippokrates-Verlag, Stuttgart, 1966.

ROYSTON, C.M.S., DOWLING, B.L., SPENCER, J.: Antrectomy with Roux and Y anastomosis in the treatment of peptic oesophagitis with stricture. Brit. J. Surg. 62 : 605, 1975.

SCOTT, H.W., WEIDNER, M.G.: Total gastrectomy with Roux and Y oesophago-jejunostomy in treatment of gastric cancer. Annals. of Surg. 143 : 682, 1956.

SELEYE, H.: Peptic haemorrhagic oesophagus. The can. med. assoc. J. 39 : 447, 1938.

SKINNER, D.B., BELSEY, R.H.R.: Surgical management of oesophageal reflux and hiatus hernia: Long-term results with 1.030 patients. J. Thorac. Cardio Vasc. Surg. 53 : 33, 1967.

SKINNER, D.B., BELSEY, R.H.R., HENDRIX, T.R., ZUIDEMA, G.D.: Gastro-esophageal reflux and hiatal hernia. Little, Brown and Company Boston, First ed. 1972.

STOL, D.W., COLLIS, J.L.: Duodeno-gastric reflux and acid secretion in patients with symptomatic hiatal hernia. Scand. J. Gastro-enterology. 9 : 97, 1974.

SVOBODA, A.S., KNAUER, M., GAMBLE, C.N., GAMBLE, C.N., SOMMERS, S.C., MONROE, L.S.: Problems in the early diagnosis of peptic oesophagitis, Gastro-Intestinal Endoscopy. 13 : 14, 1967.

WEAVER, A.W., LARGE, A.M., WALT, A.J.: Surgical management of severe reflux oesophagitis. Amer. J. Surg. 119 : 15, 1970.

WENGER, J., TROWBRIDGE, C.G.: Bile and trypsin in the stomach. Southern Med. J. 64 : 1063, 1971.

WERTHER, J.L., JANOWITZ, H.D., DYCK, W.P., CHAPMAN, M.L., RUDICK, J.: The effect of bile on electrolyte movement across canine gastric antral and fundic mucosa. Gastro-enterology. 59 : 691, 1970.

WESDORP, E., BARTELSMAN, J., PAPE, K., DEKKER, W., TYTGAT, G.N.: Oral cimetidine in reflux-oesophagitis: A double blind controlled trial. Gastroenterology. 74 : 821, 1978.

WINKELSTEIN, A.: Peptic oesophagitis J.A.M.A. 104 : 906, 1935.

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## CURRICULUM VITAE

De schrijver van dit proefschrift werd in 1946 geboren te Bolnes. De middelbare school werd bezocht in Dordrecht, alwaar hij in 1963 het diploma H.B.S.-B behaalde.
In hetzelfde jaar begon hij de medische studie aan de Rijksuniversiteit te Groningen.
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Gedurende zijn militaire dienst was hij werkzaam op de afdeling Heelkunde in het militair hospitaal A. Matthijsen te Utrecht.
In januari 1974 begon hij zijn opleiding tot chirurg in het Academisch Ziekenhuis Dijkzigt te Rotterdam.
Na inschrijving in het specialisten register in januari 1980 is hij werkzaam als chirurg bij de medische faculteit van de Erasmus Universiteit te Rotterdam.