

## MEDICAL PRACTICE

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*Hospital Topics*

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**Oesophagoscopy, Biopsy, and Acid Perfusion Test in Diagnosis of "Reflux Oesophagitis"**

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*British Medical Journal*, 1975, 1, 71-76**Summary**

The results of fiberoptic endoscopy have been assessed retrospectively in 71 patients referred for consideration of the oesophagus as the possible or probable cause of their symptoms. Gross endoscopic abnormality was uncommon but friability of the mucosa was seen in about half of the patients with typical symptoms of "reflux-pain" and a quarter of those without. The combination of radiological reflux and endoscopic abnormality—that is, true reflux oesophagitis—was seen in only a third of the patients with typical symptoms though much less commonly in those with atypical symptoms. Histological abnormality was common but did not relate well to symptom pattern. The results of the acid perfusion test were significantly related to symptom pattern though overlap was observed between the two symptomatic groups. Six of these patients had had or were awaiting surgery to correct reflux and they all had uniformly positive findings.

This study confirms the value of the acid perfusion test in clarifying the diagnosis of reflux pain, especially if the symptoms are difficult to assess. Endoscopy and biopsy added little further information of diagnostic value and could probably be reserved for the small minority of

patients who have special problems such as blood loss or dysphagia or where clarification of a radiological lesion is required.

**Introduction**

The term "reflux oesophagitis" is usually applied to a well-recognized clinical syndrome with typical symptoms and radiological evidence of gastroesophageal reflux. Many patients, however, have atypical symptoms and the radiological findings may be difficult to interpret. For example, reflux may be difficult to show even when the symptoms are typical and, conversely, the attribution of atypical symptoms to demonstrable reflux may be incorrect. These patients are often referred for endoscopic clarification. We assessed the results of endoscopy retrospectively in a group of such patients, taking into account the symptom pattern, radiological findings, and, where available, the results of the acid perfusion test and oesophageal biopsies.

**Patients and Methods**

The 71 adult patients in this survey were seen as part of a routine upper gastrointestinal endoscopy service provided by G.E.S. and J.M.T.W. from February 1971 to February 1973. They all had chronic continuous or intermittent symptoms, and attention had been directed to the oesophagus either by the symptoms or by the radiological finding of a hiatus hernia. Routine upper gastrointestinal radiology had failed to show any abnormality of the oesophagus, stomach, or duodenum apart from a sliding hiatus hernia with or without reflux. Patients with other oesophageal abnormalities such as paraoesophageal hernia, carcinoma, stricture, peptic ulcer, monilial infection, or varices were excluded.

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## SYMPTOMS

The symptoms were assessed at the time of endoscopy and the case records were also scrutinized for details of the patient's history. The symptom patterns were divided into two groups: typical and atypical. Typical symptoms consisted of pain or discomfort confined mainly or wholly to the retrosternal area associated with at least one of the following features: a relationship to posture with or without regurgitation; aggravation by swallowing—especially, hot liquids—with or without a sensation of blockage; and definite relief by antacids. Atypical symptoms included retrosternal discomfort or pain unaccompanied by any of the three features just mentioned, pain or discomfort mainly in epigastrium or abdomen, symptoms such as regurgitation or flatulence without actual discomfort, frank or suspected upper gastrointestinal blood loss, or isolated dysphagia unaccompanied by any other typical feature.

## RADIOLOGY

The available x-ray pictures and radiological reports were assessed at the time of endoscopy and the presence or absence of radiological reflux noted. Reports describing trivial or dubious abnormalities were discounted and only unequivocal evidence of reflux was accepted as positive. In the radiological department of St. Bartholomew's Hospital the routine assessment for hiatus hernia and reflux involves a downward head tilt in the prone or semiprone position. More elaborate procedures using bolsters or swallowing water after the barium are not used routinely.

Hiatus hernia was observed in 46 patients, of whom 20 had evidence of reflux. There was only one patient with reflux in the absence of a demonstrable hernia. The high incidence of hernia reflects patient selection. In line with current thinking<sup>1</sup> we assumed that the finding of hernia without reflux is of no symptomatic significance and confined our attention to the presence or absence of reflux.

## ENDOSCOPY

Initially the Olympus E.F. oesophagoscope was used, but in the second year this was replaced by the longer Olympus G.I.F.-D end-viewing instrument. The procedure was performed under sedation with intravenous diazepam (5-20 mg) after the throat had been anaesthetized with a lignocaine (2%) gargle. The entire oesophagus was examined carefully and the oesophagogastric junction was clearly identified in most cases. To reduce observer variation and error to a minimum evidence of oesophagitis was based exclusively on the presence of friability—that is, spontaneous bleeding or bleeding induced by gentle brushing. These changes were often slight and observed only in the distal few centimetres of the oesophagus but were still regarded as positive. Other changes of inflammation such as redness, oedema, granularity, and alterations of vascular pattern were considered to be unreliable as criteria in our hands and therefore disregarded. After the oesophagus had been inspected the rest of the stomach and (with the G.I.F.-D instrument) the proximal duodenum were examined to confirm the absence of other macroscopic abnormalities.

## HISTOLOGY

At least two biopsy specimens were taken from the oesophageal mucosa with endoscopic forceps. The specimens were taken from macroscopically abnormal areas if present or from different points in the distal 10 cm of an apparently normal oesophagus. Biopsies were not performed routinely at the beginning of the survey so histological information was not available on all the patients. The specimens were placed in 10% formol saline and subsequently embedded on edge in paraffin wax. Sections

5- $\mu$ m thick were stained with haematoxylin and eosin. Measurements of the height of the basal cell layer and the dermal papillae were taken using an eyepiece micrometer and expressed as a percentage of the total thickness of the mucosa. When the basal cell layer was difficult to delineate the periodic-acid Schiff stain, which left the basal cell layer unstained, was used.

The criteria of normality were those of Ismail-Beigi *et al.*<sup>2</sup> A biopsy specimen was considered normal if the basal cell layer occupied 15% or less and the dermal papillae 67% or less of the total mucosal thickness (fig. 1). Initially, if either of these

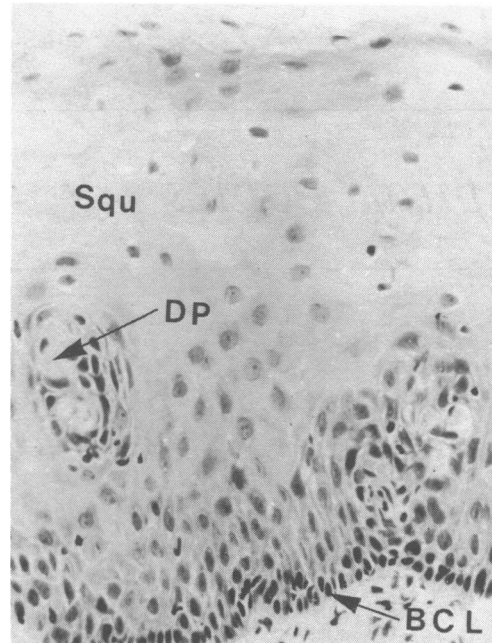


FIG. 1—Normal oesophageal mucosa. Squ = Mature squamous epithelium. DP = Dermal papillae. BCL = basal cell layer, which is here only one or two cells thick and occupies much less than 15% of the epithelium. (H. and E.  $\times$  350.)

measurements was greater than this the specimen was considered abnormal (fig. 2). In five biopsies three or fewer papillae were obtained, while in the remaining biopsies no specimen was obtained in which the basal cell height was normal in the presence of abnormal dermal papillary height. We therefore concluded that in small biopsy specimens basal cell height alone would be an accurate criterion of oesophageal abnormality. In four biopsy specimens an abnormal basal cell height was found in the presence of a normal dermal papillary height, but in three of these specimens papillae were scant.

The histological features were assessed by R. H. R. without knowledge of the patient's symptoms or endoscopic appearances.

## ACID PERFUSION TEST

The acid perfusion test was performed according to a protocol based on the method of Bennett and Atkinson.<sup>3</sup> A standard polyvinyl chloride nasogastric (Ryles) tube was passed to mid-oesophagus and the position checked radiologically. Sodium chloride 0.15 mol/l. was perfused at 10 ml/min by gravity for 10 minutes followed by hydrochloric acid 0.1 mol/l. at the same rate for 15 minutes or until symptoms were produced. If no symptoms were produced the drip rate was increased to 20 ml/min for a further 15 minutes before the result was regarded as negative. If symptoms were produced at either flow rate the infusion was replaced by sodium bicarbonate 0.1 mol/l. at the same rate. After relief of symptoms acid was infused again to check that the result was reproducible. The bottles were sus-

pended behind the patient's head so that the solutions could be changed without his knowledge. The result was considered positive if the perfusion of acid, but not saline, reproduced the major symptom(s) that the patient recognized as his own and if these were relieved by alkali. In positive cases the symptoms were usually reproduced and relieved within 5-10 minutes of starting the appropriate perfusion.

The production of trivial symptoms not previously recognized by the patient and the failure to obtain relief by alkali were regarded as negative results. Two patients experienced definite

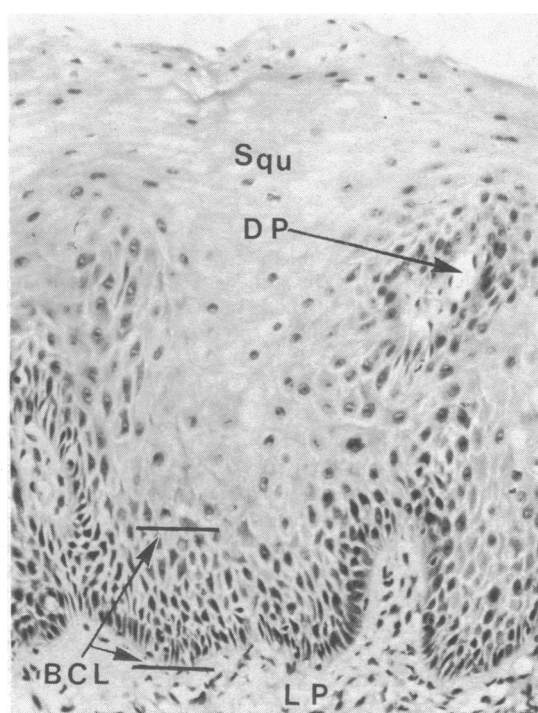


FIG. 2—Basal cell hyperplasia. Squ = Mature squamous epithelium. DP = Dermal papilla encroaching into superficial third of epithelium. BCL = Basal cell layer which is widened and occupies 22% of the total epithelium. Slight tangential cutting does not affect this figure. LP = Lamina propria. (H. and E. × 350.)

and typical heartburn, relieved by alkali, which was quite unlike their own complaints. This was termed a "positive unrelated" response by Bernstein *et al.*,<sup>4</sup> and we regarded it as a negative response. In both these patients other findings were uniformly negative. The procedure was abandoned in four subjects because they were unable to tolerate it, and the results were disregarded in two others who were quite unable to give a clear description of their symptoms.

These perfusion tests were not performed by the endoscopist, but the results were assessed afterwards by him. They were performed by junior hospital staff or medical students according to a strict protocol to obviate observer bias.

STATISTICS

The results were analysed by the  $\chi^2$  test ( $3 \times 2$  contingency

tables), Yates's  $\chi^2$  test ( $2 \times 2$  contingency tables  $N > 50$ ), or Fisher's test ( $2 \times 2$  contingency tables  $N < 50$ ).<sup>5</sup> P values equal to or less than 0.05 are quoted. Where no P value is stated it can be assumed that it was greater than 0.05.

Results

Endoscopy

The incidence of radiological reflux or endoscopic abnormality or both was appreciably higher in the patients with typical symptoms, but this was statistically significant for reflux only ( $P = 0.01$ ; table I). Only one-third of patients with typical symptoms had both reflux and endoscopic change—that is, true reflux oesophagitis. The endoscopic abnormalities were mild in most cases, only six patients showing more gross and extensive abnormality. Two patients with atypical symptoms had gross oesophagitis. They had previously had mild dyspepsia but presented acutely with frank upper gastrointestinal haemorrhage and little or no pain. If reflux and endoscopic change were considered together in individual patients there was a statistically significant separation of the two symptomatic groups ( $P < 0.01$ ; table I).

TABLE II—Relation between Presence or Absence of Reflux and Endoscopic Appearances in 71 Patients. Results are Numbers of Patients

	Endoscopic Abnormalities	No Endoscopic Abnormalities	Total
Reflux .. ..	11	10	21
No reflux .. ..	14	36	50
Total	25	46	71

Acid Perfusion Test

Successful acid perfusion tests were performed on 48 of the 71 patients (table III). In these patients the incidence of reflux and endoscopic abnormality in the two symptomatic groups was similar to that shown in the whole group. A positive perfusion test result was obtained in most patients with typical symptoms and in a few of those without. Statistically, the results of the acid perfusion test were significantly related to symptom pattern and endoscopic abnormality ( $P < 0.01$ ) (tables III and IV) but not to reflux. If endoscopy and acid perfusion test were considered together in individual patients the symptomatic groups were significantly separated ( $P < 0.01$ ; table III).

Histological Evidence

Sixty-four interpretable biopsy specimens were available from 36 patients, and 16 further specimens were uninterpretable (five were fragmented or inadequate, five were gastric, four had no basal layer, one was ulcerated, and one was only a blood clot). Lamina propria was present in material from 26 patients.

There was a much greater incidence of abnormalities in biopsy specimens from patients with typical symptoms, but this failed to reach statistical significance (table V) though significance was reached in the smaller group in which the acid perfusion

TABLE I—Relation of Symptom Patterns to Endoscopic Appearances and Presence of Radiological Reflux in 71 Patients. Results are Numbers of Patients

	No. of Patients	Reflux*		Endoscopic Appearances		Reflux and Endoscopic Abnormalities†		
		Present	Absent	Abnormal	Normal	Both Present	One Present	Neither Present
Typical symptoms .. ..	26	13	13	13	13	9	8	9
Atypical symptoms .. ..	45	8	37	12	33	2	16	27

\* $P < 0.01$ . † $P < 0.01$ .

TABLE III—Relation of Symptom Patterns to Endoscopic Appearances, Presence of Radiological Reflux, and Results of Acid Perfusion Test in 48 Patients. Results are Numbers of Patients

	No. of Patients	Reflux	No Reflux	Endoscopic Appearances*		Perfusion Test Results†		Endoscopy Findings and Perfusion Test Result‡		
				Abnormal	Normal	Positive	Negative	Both Positive	One Positive	Neither Positive
Typical symptoms ..	19	10	9	9	10	14	5	8	7	4
Atypical symptoms ..	29	7	22	5	24	5	24	2	6	21

\*P = 0.05. †P &lt; 0.01.

TABLE IV—Relation between Perfusion Test Result, Endoscopic Appearances, and Presence or Absence of Reflux in 48 Patients. Results are Numbers of Patients

Perfusion Test Result	No. of Patients	Reflux	No Reflux	Endoscopic Appearances*	
				Abnormal	Normal
Positive ..	19	8	11	10	9
Negative ..	29	9	20	4	25

\*P &lt; 0.01.

test was also carried out ( $P < 0.05$ ; see table VII). Of the 11 subjects with normal histological appearances two had typical symptoms, one was endoscopically abnormal, one had an abnormal acid perfusion test result, but none had reflux. The relationship between histological appearances and both endoscopic abnormality ( $P < 0.05$ ) and radiological reflux ( $P = 0.05$ ) reached statistical significance (table VI).

Submucosal polymorphs were present in 10 out of 18 specimens with basal cell hyperplasia in which lamina propria was present. In three out of seven further abnormal biopsy specimens, in which no lamina propria was present, polymorphs could be identified in the dermal papillae. By contrast, in none of the 11 normal specimens were polymorphs seen in lamina propria if present or in the dermal papillae. This relationship was statistically significant ( $P < 0.01$ ).

When gastric mucosa was obtained on biopsy there is the possibility that ulcerated oesophageal mucosa had become re-epithelialized from the gastric mucosa. While this would indicate previous oesophageal disease we ignored it here. Similarly, the single ulcerated biopsy specimen was disregarded as its exact site of origin was not accurately known and no adjacent mucosa was present.

#### Acid Perfusion Test and Histology

Interpretable information about both the acid perfusion test and histological features was available in 27 patients and the distribution of abnormal findings was similar to that in the previous groupings (table VII). Of the 11 patients with typical symptoms the acid perfusion test gave a positive result in most and the histological appearances were abnormal in all. The relation between symptom pattern and histology was statistically significant in this subgroup ( $P < 0.05$ ), whereas that between symptoms and acid perfusion was not. Only one out of 13 patients with a positive acid perfusion test result had normal histological appearances. This relationship was not statistically significant (table VIII).

Cumulative evidence in relation to symptoms was based on

TABLE VI—Relation between Histological Appearances, Presence or Absence of Reflux, and Endoscopic Appearances in 36 Patients. Results are Numbers of Patients

Histological Appearances	No. of Patients	Reflux*	No Reflux	Endoscopic Appearances†	
				Abnormal	Normal
Abnormal ..	25	8	17	12	13
Normal ..	11	0	11	1	10

\*P = 0.05. †P &lt; 0.05.

three criteria (endoscopy, acid perfusion, and histology) and is shown in table VII. The difference between patients positive on two or three criteria and those positive on one or none was statistically significant ( $P < 0.05$ ).

#### Discussion

Though the oesophagus was suspected as a possible or probable cause of symptoms in all these patients only six (8.4%) had endoscopic evidence of severe and extensive oesophagitis. Of these six patients two presented with upper gastrointestinal haemorrhage without pain and minimal previous clinical evidence of oesophageal reflux. Lesser endoscopic abnormalities were observed in about half the patients with typical symptoms of reflux oesophagitis, but the endoscopic criteria used were possibly too strict and might have excluded slighter but significant abnormalities. Other authors describe changes such as redness and oedema as indicative of mild oesophagitis.<sup>6,7</sup> We considered that such criteria would be liable to considerable observer variation and error but know of no formal study in which this has been assessed. By analogy with studies of proctitis<sup>8</sup> we considered that friability would be the most objective criterion. Grosser and unequivocal evidence of oesophagitis, such as stricture or frank ulceration, was not seen in this series.

Other reports indicate a higher incidence of endoscopic abnormality in patients with typical reflux heartburn,<sup>2-4,7</sup> but in these and other studies it is apparent that symptoms and endoscopic findings do not always correlate.<sup>9,10</sup> The relation probably depends on the chronicity and severity of symptoms rather than their quality. In a series of 76 patients coming to operation, presumably with chronic unremitting symptoms, Cocco and Brantigan<sup>11</sup> observed endoscopic abnormality in 69. In six of our patients coming to surgery all had endoscopic abnormality.

The histological criteria of oesophagitis were until recently the classical ones of oedema and hyperaemia with an inflammatory infiltrate in the lamina propria and possibly subsequent fibrosis.<sup>12</sup> As a mononuclear infiltrate is normally seen in the

TABLE V—Relation of Symptom Patterns to Endoscopic Appearances, Presence of Radiological Reflux, and Histological Appearances in 36 Patients. Results are Numbers of Patients

	No. of Patients	Reflux	No Reflux	Endoscopic Appearances		Histological Appearances		Endoscopic and Histological Appearances		
				Abnormal	Normal	Abnormal	Normal	Both Abnormal	One Abnormal	Neither Abnormal
Typical symptoms ..	14	5	9	6	8	12	2	6	6	2
Atypical symptoms ..	22	3	19	7	15	13	9	6	8	8

TABLE VII—Relation of Symptom Patterns to Endoscopic Appearances, Presence of Radiological Reflux, Acid Perfusion Test Results, and Histological Appearance in 27 Patients. Results are Numbers of Patients

	No. of Patients	Reflux	No Reflux	Endoscopic Appearances		Perfusion Test Results		Histological Appearances*		Perfusion Test Results and Endoscopic and Histological Findings			
				Abnormal	Normal	Positive	Negative	Abnormal	Normal	All Positive	Two Positive	One Positive	None Positive
Typical symptoms .. ..	11	5	6	5	6	8	3	11	0	4	5	2	0
Atypical symptoms .. ..	16	3	13	4	12	5	11	9	7	2	3	6	5

\*P &lt; 0.05.

TABLE VIII—Relation between Acid Perfusion Test Result and Histological Appearances in 27 Patients. Results are Numbers of Patients

Perfusion Test Result	Histological Abnormalities	No Histological Abnormalities	Total
Positive .. ..	12	1	13
Negative .. ..	8	6	14
Total	20	7	27

lamina propria the presence of polymorphs is a useful criterion of inflammation. There may also be ulceration of the surface epithelium, with exposure of the capillary channels in the dermal papillae, accounting for both the hyperaemia and haemorrhage seen endoscopically.

Ismail-Beigi *et al.*<sup>2</sup> described changes in the squamous epithelium in 28 out of 33 patients with typical reflux heartburn compared with two out of 21 controls (see Methods). They obtained large biopsy specimens by suction, but we were interested in applying these criteria to specimens obtained by fibre-optic instruments. Endoscopic biopsy specimens are small, difficult to orientate, and often contain little lamina propria. Other workers have concluded that they are generally too small for conventional histological interpretation.<sup>13</sup> In our study, using criteria based on thickness of basal cell layer, 25 out of 36 patients were considered to have abnormal biopsy specimens. Of these 20 had other evidence of oesophageal disease (on endoscopy, radiology, acid perfusion test, or a combination of these). Of the remaining 11 patients with normal specimens none had radiological reflux, one had a positive acid perfusion test result, and one had endoscopic abnormality. The latter may reflect the sampling problem found by Ismail-Beigi *et al.*<sup>2</sup> in 25% of paired samples. In 21 patients of our series in whom two or more satisfactory biopsy specimens were obtained only two had discrepant histological appearances. Significant relationships were found between histological abnormality and endoscopic appearance, radiological reflux, and the presence of polymorphs in the lamina propria or dermal papillae. We did not consider it justifiable to submit normal volunteers to endoscopy to obtain a strictly normal group of oesophageal biopsy specimens.

Basal cell hyperplasia seems, therefore, to be a sensitive index of oesophageal abnormality as judged by several other criteria. It may, however, be too sensitive and indicate trivial or intermittent reflux of no clinical importance. In the present series five out of 25 patients with abnormal specimens had no other positive feature suggestive of oesophageal disease, and yet in all the basal cell layer accounted for at least 20% of mucosal thickness and in four polymorphs were present in the lamina propria. No readjustment of the criteria of Ismail-Beigi *et al.*<sup>2</sup> could be made to classify these as normal without invalidating the relationship between histology and clinical features in many more patients. On the other hand, in the absence of basal cell hyperplasia it seems unlikely that there will be other good evidence of oesophageal abnormality. In view of evidence suggesting that cigarette smoking might be a common cause of oesophageal reflux<sup>14</sup> a smoking history was obtained retrospectively in 35 of the 36 patients with satisfactory histological specimens. Of the 25 with abnormal histological appearances 14 were regular smokers and 11 were non-smokers, and out of the 10 patients

with normal appearances four were smokers and six were non-smokers. (Two patients who smoked very rarely were grouped as non-smokers.) Though abnormal appearances were more common in smokers than in non-smokers the difference was not significant ( $P > 0.05$ ).

The acid perfusion test has been widely used in the differential diagnosis of oesophageal and cardiac pain. In several series there has been an extremely close relationship between the results of acid perfusion and symptoms in patients with typical heartburn compared with asymptomatic controls.<sup>2-4 15</sup> On the other hand Spencer<sup>16</sup> has reported a potentially misleading low incidence of positive results in patients with a variety of other upper gastrointestinal disorders, but he did not relate these results to their actual symptoms. Earlam<sup>17</sup> has claimed that the epigastric pain of duodenal ulcer can be reproduced by acid perfusion of the lower oesophagus. We regarded the result as positive only if the patient's own symptoms were reproduced, and none of these patients had evidence of active peptic ulcer or other conditions at the time of the investigation. The perfusion test was better than the other criteria in distinguishing patients with typical and atypical symptoms, in that a clear majority of patients with typical symptoms had a positive test result and a clear majority of those with atypical symptoms had a negative result. On the other hand, only half the patients with a positive test result had abnormal endoscopic appearances, whereas most in a smaller group of patients had abnormal histological appearances. This latter finding is difficult to interpret because more than half the patients with a negative perfusion test result also had abnormal histological appearances. Of the five patients with atypical symptoms and a positive perfusion test two had endoscopic evidence of oesophagitis and, symptomatically, three had retrosternal pain and two had epigastric pain associated with regurgitation. Thus, with a few exceptions a positive perfusion test result was associated with symptomatic or other evidence of acid reflux. On the other hand, a negative result may not exonerate the oesophagus, as indicated by the five patients with typical symptoms and a negative result; two had radiological reflux, one other had abnormal endoscopic appearances, and only two had negative findings on all criteria.

We did not assess reflux formally and the evidence was based solely on routine radiological procedures performed by a number of radiologists. There was a low incidence of reflux in the series (21 out of 71 patients) and among the patients who were positive on the other criteria (typical symptoms, histology, acid perfusion, etc), there was no incidence of reflux greater than 50%. On the other hand, the incidence of reflux was very low among patients who were negative on the various criteria. All the patients who had had or were awaiting surgery had free radiological reflux. Probably routine radiological examinations as performed in these patients underestimated the true incidence of reflux, because in other series reflux has been shown to be present by pH probe techniques in most patients with typical symptoms or a positive acid perfusion test result or both.<sup>2 15 18</sup> Negative findings on radiology should not refute a clinical diagnosis of reflux pain especially if the diagnosis is supported by a positive perfusion test result, and repeat radiology may show the reflux if special attention is paid to its elicitation.

Considering all the evidence our results show that in patients with the typical reflux heartburn syndrome macroscopic evidence of oesophagitis is often minimal or lacking altogether. The acid

