The gastric mucosa in anaemia in Punjabis

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Part I In iron-deficiency anaemia

EDITORIAL SYNOPSIS The authors carried out gastric biopsies and augmented histamine tests in patients in the Punjab with iron-deficiency anaemia. An increased incidence of atrophic gastritis and impairment of gastric secretion was observed only in the patients in whom there had been no obvious blood loss and it is suggested that dietary insufficiency or intestinal malabsorption may be more important than iron deficiency in the aetiology of atrophic gastritis.

Although the aetiology and pathological significance of chronic atrophic gastritis is still unknown, the incidence of the disorder in normal subjects and in a variety of pathological conditions is becoming known. Coghill (1960), in a full review of the subject, discussed the possible relationship between gastritis and hypochromic anaemia. It has been suggested that the gastric mucosal lesion may result from iron deficiency (Witts, 1956; Badenoch, Evans, and Richards, 1957), but this view is not supported by Foy and Kondi (1960), who found that achlorhydria was rare in patients with severe iron deficiency secondary to hookworm infestation, or by Coghill (1960), who showed that atrophic gastritis occurred more frequently in patients with 'idiopathic' hypochromic anaemia, that is, in whom there was no evidence of blood loss or exceptionally low iron intake to account for the iron deficiency, than in patients suffering from chronic haemorrhage.

Many patients with iron-deficiency anaemia attend Brown Memorial Hospital, the chief causes being hookworm infestation, dietary deficiency, and gynaecological disorders. In view of the high incidence of this type of anaemia, it was decided to extend the routine haematological investigation of these patients to include biopsy of the gastric mucosa in an attempt to establish the incidence of gastritis and gastric atrophy, and also to see if there were any clinico-pathological associations which might shed further light on the pathogenesis of the gastric mucosal lesion.

MATERIAL AND METHODS

Seventy patients with hypochromic anaemia were studied. The cause of the anaemia in 33 was hookworm infestation

(*Ancylostoma duodenale*), while in 37 there was no evidence of hookworm. Males greatly predominated in the group with hookworm infestation, while the sex ratio was the reverse in the others (Table VI). Fourteen of the non-hookworm group gave a history of bleeding, two male patients had bleeding haemorrhoids, while 12 females had menorrhagia. In the remaining 23 patients no evidence of blood loss could be found. In addition to a detailed history and physical examination, including proctoscopy, pelvic examinations were performed in all parous women. Chest screening was done in every case to exclude active pulmonary tuberculosis.

STOOL EXAMINATIONS The stools were examined for the presence of hookworm ova on three occasions and persistently negative tests were taken as evidence that the anaemia was not due to hookworm infestation. The possibility of a vermifuge having been taken before admission to hospital was excluded, as far as possible, by means of careful enquiry. The stools were also repeatedly tested for occult blood in an attempt to exclude the possibility of bleeding from the upper alimentary tract.

DIET All the patients were drawn from the poorer social classes; on the basis of the amount of meat, milk, wheat, pulses, and vegetables consumed, the diet was classified as 'very poor' in 22 and only 'fair' in the remaining 48. While only a third claimed that they were vegetarians, very few of the remainder could afford to eat meat regularly. Table I shows the distribution of the patients according to diet and blood loss.

HAEMATOLOGY Standard haematological techniques were employed (Dacie, 1956) including careful scrutiny of peripheral blood films and bone marrow smears in all cases. No evidence of macrocytosis or megaloblastosis was present in any of the patients in the series. Haemoglobin levels ranged from 2 to 10 g. %, the average for the group being 6 g. %.

TABLE I

RELATIONSHIP OF INCIDENCE OF ATROPHIC
GASTRITIS TO DIET AND BLOOD LOSS

Diet	No. of Patients	No. with Atrophic Gastritis	Percentage Incidence of Atrophic Gastritis
<i>Fair</i>			
Blood loss	32 (22 hook-worm)	2	6.25
No blood loss	16	6	37.5
<i>Poor</i>			
Blood loss	15 (11 hook-worm)	3	20.0
No blood loss	7	3	42.8

SERUM IRON These estimations, using Ramsay's method (1957), were begun during the later part of the study and were done in 30 instances only.

GASTRIC ACID Gastric acidity was determined by means of the augmented histamine test (Kay, 1953) with the minor modifications of Bock, Richard, and Witts (1963b) and using 50 mg. of pheniramine maleate to counteract the effects of the large dose of histamine. The gastric juice was obtained by means of continuous hand suction, and the acidity expressed as the number of milliequivalents of HCl secreted in one hour following the administration of histamine (0.04 mg. per kilogram of body weight).

GASTRIC MUCOSA Gastric mucosal biopsies were obtained in all of the 70 patients by means of Wood's flexible peroral suction biopsy tube (Wood, Doig, Motteram, and Hughes, 1949), and in most cases two biopsies were taken from the body of the stomach of each patient. No case of significant bleeding as a result of the biopsy was encountered. The biopsies were orientated, mucosal surface upwards, on blocks of wood, fixed in 10% formalin, cut at right angles, and stained in the routine manner with haematoxylin and eosin. The sections were examined independently by two pathologists, neither of whom was aware of the haemoglobin level or acid output of the patients. Not only was there almost complete agreement between the two observers with regard to the degree of gastritis present, but where two biopsies were taken from the same patient, similar findings were present in approximately 90% of the cases. The classification of Bock, Arapakis, Witts, and Richards (1963) was used to determine the presence of superficial and atrophic gastritis, but gastric atrophy without cellular infiltration was not seen in any biopsy examined in the course of this investigation. The degree of glandular atrophy was variable, but even when the atrophy was very severe, there was marked infiltration with inflammatory cells, chiefly lymphocytes and plasma cells, but polymorphonuclear leucocytes were also seen.

RESULTS

GASTRIC MUCOSA Gastric mucosal abnormalities (Table II) were found in 43 patients (61.4 %). The

TABLE II

HISTOLOGY OF THE GASTRIC MUCOSA

Histology	No. of Cases	Percentage Incidence
Normal	27	38.6
Superficial gastritis	29	41.4
Atrophic gastritis	14	20.0

lesion was superficial gastritis in 29 (41.4%) and atrophic gastritis in 14 (20%). The atrophic gastritis was of a moderate degree in six instances and severe in eight. Gastric atrophy without cellular infiltration was not found; in fact, inflammatory cells were most numerous in those biopsies which showed the severest degrees of glandular atrophy.

In Table III these results are rearranged according to the presence or absence of hookworm infestation. While the incidence of superficial gastritis was almost the same in the two groups, there was a marked difference in the incidence of atrophic gastritis; two of the 33 patients with hookworm and 12 of the 37 without hookworm had atrophic gastritis. This is a highly significant difference ($\chi^2 = 5.98$ with Yates' correction, $P < 0.02$).

TABLE III

RESULTS ACCORDING TO PRESENCE OF
HOOKWORM INFESTATION

Histology	33 Cases with Hookworm	37 Cases without Hookworm
Normal	17 (51.5%)	10 (27.0%)
Superficial gastritis	14 (42.4%)	15 (40.5%)
Atrophic gastritis	2 (6.0%)	12 (32.4%)

Fourteen of the patients without hookworm infestation gave a history of bleeding from the reproductive tract, or from haemorrhoids, and this was severe enough to account for their anaemia. These, together with the hookworm group, form a group of 47 patients whose anaemia was wholly or partly due to blood loss, leaving 23 in whom there was no demonstrable source of blood loss to account for the anaemia. Table IV shows the results rearranged according to the presence or absence of blood loss. It is apparent that there was a marked difference in the incidence of gastritis between the two groups; five of the 47 with blood loss and nine of the 23 without blood loss had atrophic

TABLE IV

RESULTS ACCORDING TO PRESENCE OF
BLOOD LOSS

Histology	47 Cases with Blood Loss	23 Cases without Blood Loss
Normal	20 (42.5%)	7 (30.4%)
Superficial gastritis	22 (46.8%)	7 (30.4%)
Atrophic gastritis	5 (10.6%)	9 (39.1%)

gastritis ($\chi^2 = 5.54$ with Yates' correction, $P < 0.02$).

GASTRIC ACIDITY Eleven of the cases of atrophic gastritis had complete achlorhydria, but the maximal acid output in the remaining three was 8.8, 2.6, and 3.3 mEq. Three patients whose gastric mucosa was either normal or showed superficial gastritis secreted less than 1 mEq. of acid. Table V shows that the relationship between the acid output and the histology of the gastric mucosa was similar to that found by Bock *et al.* (1963b), whose results are also shown in Table V.

TABLE V
RELATIONSHIP OF ACID OUTPUT TO HISTOLOGY
OF THE GASTRIC MUCOSA

Histology	Present Study		Bock <i>et al.</i> (1963b)	
	Hb (g. %)	Maximal Acid Output (mEq.)	Hb (g. %)	Maximal Acid Output (mEq.)
Normal mucosa	6.8	7.6	13.8	22.00
Superficial gastritis	6.4	8.1	12.1	10.00
Moderate atrophic gastritis	5.9	2.3	12.9	5.54
Severe atrophic gastritis	4.9	0.5	—	—
Gastric atrophy	—	—	11.9	0.02

Table VI shows that the acid output in patients with hookworm infestation was considerably higher than in those without hookworm, and Table VII shows that, in the total haemorrhagic group, the acid output was higher than it was in the group whose anaemia was 'idiopathic'. Since there was little difference between the two groups with regard to haemoglobin or serum iron levels, the difference in acid output must therefore be due to some other

TABLE VIII

INCIDENCE OF ATROPHIC GASTRITIS IN NORMAL AND DUODENAL ULCER SUBJECTS COMPARED WITH INCIDENCE IN HAEMORRHAGIC AND 'IDIOPATHIC' IRON-DEFICIENCY ANAEMIA

	No.	Maximal Acid Output (mEq.)	No. with Atrophic Gastritis
Normal subjects	29	12.0	0 (0)
Duodenal ulcer	18	19.0	0 (0)
Iron-deficiency anaemia (haemorrhagic)	47	7.7	5 (10.6%)
Iron-deficiency anaemia (idiopathic)	23	4.3	9 (39.1%)

factor. The results of augmented histamine tests carried out by the authors on 29 normal subjects and 18 patients suffering from duodenal ulceration are shown in Table VIII where they are compared with the results of the present study. While superficial gastritis was found in three normal subjects and six duodenal ulcer patients, atrophic gastritis was not found in either group.

DISCUSSION

The results reported here confirm the suggestion of Coghill (1960) that atrophic gastritis is more common in patients with 'idiopathic' iron-deficiency anaemia, that is, in whom there is no history of blood loss to account for their anaemia, than it is in patients whose anaemia is due to blood loss. In the present study, the degree of anaemia did not appear to account for the difference in the incidence of atrophic gastritis as the haemoglobin levels of the two groups were almost the same, nor did the degree of iron deficiency appear to be a factor, as the serum iron levels were similar in the two groups.

TABLE VI

INCIDENCE OF ATROPHIC GASTRITIS, LEVELS OF GASTRIC ACID, SERUM IRON AND HAEMOGLOBIN, AGE, AND SEX IN RELATION TO HOOKWORM INFESTATION

	No.	Average Age	Male	Female	Average Haemoglobin (g. %)	Average Serum Iron (μ g. per 100 ml.)	Maximal Acid Output (mEq.)	Incidence of Atrophic Gastritis
Hookworm	33	35	27	6	6.1	34	8.4	2 (6.06%)
No hookworm	37	32	7	30	5.9	34	4.9	12 (32.4%)

TABLE VII

INCIDENCE OF ATROPHIC GASTRITIS, LEVELS OF GASTRIC ACID, SERUM IRON AND HAEMOGLOBIN, AGE, AND SEX IN RELATION TO BLOOD LOSS

	No.	Average Age	Male	Female	Average Haemoglobin (g. %)	Average Serum Iron (μ g. per 100 ml.)	Maximal Acid Output (mEq.)	Incidence of Atrophic Gastritis
Blood loss	47	34	29	18	5.0	35	7.7	5 (10.6%)
No blood loss	23	32	5	18	6.2	33	4.3	9 (39.1%)

It was recognized that the duration of the anaemia rather than the presence or absence of blood loss might be responsible for the difference in the incidence of atrophic gastritis, but the duration of symptoms resulting from anaemia was an unreliable indication of the duration of the anaemia. Some patients, who were known to have been severely anaemic for years, had no symptoms apart from mild tiredness, while others, with a short history of bleeding, complained of breathlessness and palpitation. Since koilonychia and glossitis are found in 15 to 20% of cases of severe iron-deficiency anaemia, in temperate zones at least (Whitby and Britton, 1957), these epithelial changes were considered to be more reliable indications of the duration of the anaemia than the histories of the patients, and were present in 32 of 47 (68.0%) with blood loss and in 14 of 23 (60.8%) without blood loss. This seems to indicate that the duration of the anaemia, as judged by the presence or absence of these epithelial changes at least, was not solely responsible for the difference in the incidence of atrophic gastritis.

Floch and Thomassen (1963), reporting on the gastric mucosa in 10 patients with hookworm, pointed out that, while iron deficiency cannot be ruled out as a causal factor in the pathogenesis of chronic atrophic gastritis, the fact that gastritis is common in tropical sprue and schistomomiasis, both prevalent in Puerto Rico where the study was done, suggests that other endemic factors or nutritional deficiency must be considered as possible causes of the gastric mucosal lesion.

While accurate evaluation of the diets of the patients in the present study was not attempted, it was not difficult to recognize very poor diets, and in these patients, the incidence of gastritis tended to be higher (Table I) and the degree of atrophy more severe, but numbers are too small for statistical evaluation.

Foy and Kondi (1958) and Jacobs (1963) have commented upon the rarity of koilonychia and glossitis in severe iron deficiency occurring in Africa, and they also comment upon the rarity of dysphagia in iron deficiency in that Continent. In the Punjab, dysphagia is seldom a presenting symptom, but koilonychia and glossitis are by no means rare, and occurred in 46 of the 70 patients (65.7%) in the present series.

In addition to these epithelial changes 11 patients had abnormal pigmentation of the nails, the dorsum of the hands and the feet, and of these, three had the skin changes of pellagra. Bisht and Singh (1962), commenting on pigmentation of the nails in South Indian patients, considered this to be a reliable sign of nutritional deficiency, and Baker, Ignatius, Johnson, and Vaish (1963) described hyperpigmen-

tation of the skin in South Indians who were deficient in vitamin B₁₂. In the present study, four of the eight biopsies showing severe atrophic gastritis were taken from patients who had abnormal pigmentation of the skin; in one it was confined to the dorsum of the hands, while the remaining three had the symmetrical pigmented skin lesions on the wrists and ankles which are typical of pellagra. These three patients, because of poverty, were accustomed to eat maize instead of wheat throughout the year, with the exception of a few weeks following the wheat harvest.

Multiple nutritional deficiencies are common in the Punjab where many people are vegetarians or cannot afford to eat meat, and whose diets are, in many cases, no more than marginally adequate. Malabsorption, caused by structural or functional abnormalities of the small intestine, is also common, and it is possible that a more important factor than iron deficiency in the pathogenesis of chronic atrophic gastritis in this area at least, is nutritional deficiency, due in some instances to dietary deficiency alone, but in many cases, aggravated by disease of the small intestine.

SUMMARY

The results of gastric mucosal biopsies from 70 patients suffering from iron-deficiency anaemia are reported. Thirty-three had hookworm infestation, 14 gave a history of bleeding from the reproductive tract or from haemorrhoids, and in the remaining 23 patients, there was no history of blood loss to account for the anaemia. Gastric atrophy with little or no inflammatory cell infiltration was not found in any patient in the study, and the incidence of atrophic gastritis was significantly greater in this last group, whose anaemia could be termed 'idiopathic', than it was in those patients in whom there was evidence of blood loss. The ages of the patients in the two groups, haemoglobin levels, serum iron levels, and incidence of koilonychia and glossitis were practically identical, and the suggestion is made that nutritional deficiency, due to inadequate diet alone, or conditioned by disease of the small intestine, is a more important factor than iron deficiency in the pathogenesis of chronic atrophic gastritis.

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Part II In megaloblastic anaemia

EDITORIAL SYNOPSIS There is a relatively high incidence of atrophic gastritis in patients in the Punjab with megaloblastic anaemia. No correlation was found with age, degree of anaemia, steatorrhoea, iron deficiency, or response to vitamin B₁₂ and folic acid.

Nutritional deficiency, secondary to inadequate diet or conditioned by structural or functional disease of the small intestine, is the chief cause of the megaloblastic anaemias seen in the Punjab. It was therefore decided to examine the gastric mucosa of patients with megaloblastic anaemia (excluding that associated with pregnancy or the puerperium) in order to establish the incidence of atrophic gastritis in these cases, and to see if gastric atrophy, without marked cellular infiltration, occurred in this type of anaemia.

MATERIAL AND METHODS

Gastric mucosal biopsies were obtained from 30 patients with megaloblastic anaemia; there were 17 males and 13 females and their clinical data and laboratory findings are set out in Tables I and II.

DIET An assessment of the diets of the patients was made on the basis of the amount of meat, milk, wheat, pulses, and vegetables consumed.

NUTRITIONAL DEFICIENCY SIGNS Each patient was examined for koilonychia, glossitis, and abnormal pigmentation of the skin. This is frequently seen in patients who have other stigmata of nutritional deficiency, and consists of dark brown pigmentation of the hands, particularly over the interphalangeal joints. Baker, Ignatius, Johnson, and Vaish (1963) described this type of brown pigmentation of the skin in South Indian patients who had vitamin B₁₂ deficiency.

BLOOD LOSS Particular attention was paid to any history of blood loss; proctoscopy was performed to exclude haemorrhoids, and pelvic examinations were done in all parous women.

STOOL EXAMINATIONS The stools were examined on several occasions for occult blood and the ova of hookworm.

FAECAL FAT EXCRETION This was estimated in every case, using the method of van de Kamer, ten Bokkel Huinink, and Weyers (1949). The tests were performed on a three-day collection of stool, while the fat content of the diet was 50-100 g. per day. The results were expressed as a daily average, and steatorrhoea was considered to be present when the daily excretion exceeded 7 g.

RADIOLOGY Active pulmonary tuberculosis was excluded by fluoroscopy in all patients, and barium studies of the small intestine were done in the 15 patients who had gastrointestinal symptoms, which ranged from vague central abdominal discomfort to colicky pain suggestive of subacute obstruction in one case.

HAEMATOLOGY Standard haematological techniques were employed (Dacie, 1956). The diagnosis of megaloblastic anaemia was established on the basis of a macrocytic peripheral blood and a megaloblastic bone marrow. Peripheral blood films were carefully scrutinized for the presence of hypochromia of the red cells.

SERUM IRON This was estimated in seven instances only, using Ramsay's method (1957).