

## Autoimmunity in Iron-deficiency Anaemia\*

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*Brit. med. J.*, 1964, 1, 1349-1350

There is recent evidence to suggest that an autoimmune mechanism may be associated with the gastric mucosal changes found in pernicious anaemia. Markson and Moore (1962) and Irvine *et al.* (1962) found a complement-fixing antibody to gastric mucosa in the blood of 42% and 75% respectively of their patients with pernicious anaemia; and Taylor *et al.* (1962), using an immunofluorescent technique, demonstrated an organ-specific autoantibody in 85% of 100 patients. These last workers also showed that the antigen was localized in the cytoplasm of the parietal cells, and was recoverable in the "microsomal" fraction of homogenized gastric mucosa. The autoantibody to gastric parietal cells was shown to be quite distinct from the antibody to intrinsic factor first described by Taylor (1959).

These studies have been extended to include observations on patients with iron-deficiency anaemia. Markson and Moore (1962), using a complement-fixation test in 34 patients with iron-deficiency anaemia, found that 17.6% of these had gastric autoantibodies; there were no positive tests in 34 matched control subjects. Gastric achlorhydria is twice as common in patients with iron-deficiency anaemia as in normal subjects, and four times as common in patients under 50 years (Joske *et al.*, 1955; Badenoch *et al.*, 1957). There is an increased incidence of chronic gastritis in iron-deficiency anaemia, and not infrequently the mucosal changes are closely similar to those found in pernicious anaemia (Davidson and Markson, 1955). Furthermore, a good correlation exists between the presence of a histamine-fast achlorhydria and the finding of a severe degree of chronic gastritis (Bock *et al.*, 1963). It was for these reasons that we have investigated by an immunofluorescent test the incidence of gastric autoantibodies in the sera of 64 patients with iron-deficiency anaemia, and have related the occurrence of positive results to the presence or absence of a histamine-fast achlorhydria.

### Materials and Methods

**Antibody Test.**—An immunofluorescent "sandwich" technique closely similar to that used by Taylor *et al.* (1962) was employed to detect parietal-cell antibody in the patient's serum. Gastric mucosa was obtained from the body of the stomach of patients undergoing operation for duodenal ulcer and cryostat sections were prepared from the fresh unfixed material. Each section was treated at room temperature with a 1 in 4 dilution of the patient's serum (30 minutes), buffered saline (10 minutes with shaking), a fluorescein-conjugated rabbit antibody to human  $\gamma$ -globulin (30 minutes), buffered saline (10 minutes with shaking), and after mounting in buffered glycerol the sections were examined by ultra-violet microscopy. Distinct green fluorescence of the parietal cells was regarded as indicative of antibody in the serum under examination. Although the results were in general clear-cut, very faint green fluorescence of parietal cells was encountered with some sera, both of iron-deficient and of control subjects, and was regarded as negative. A study of the serum of patients with pernicious anaemia

(Adams *et al.*, 1964) showed the test to give closely similar results to those reported by Taylor *et al.* (1962).

The patients investigated included 64 with iron-deficiency anaemia and 64 non-anaemic "control" subjects, who were healthy individuals, although some had recently sustained a fracture. The groups were matched for age and sex.

Thirty-three of the iron-deficiency-anaemia patients had complete achlorhydria and 31 had acid in the gastric juice. A patient was regarded as having histamine-fast achlorhydria when, in response to a maximum stimulating dose of histamine (0.04 mg./kg. body weight) (Kay, 1953), the pH of the gastric juice did not fall below 6.4. In 10 patients who were intolerant of intubation acid secretion was assessed by the "diagnex" tubeless technique following administration of histamine and an antihistamine as for Kay's test. The results of this method correlate well with those of the Kay test (Bock and Witts, 1961).

The diagnosis of iron-deficiency anaemia in each case was established by the peripheral blood picture, absence of stainable iron in a normoblastic bone-marrow, a characteristically low serum-iron level and high iron-binding capacity, and complete remission of the anaemia in response solely to iron therapy. The known aetiological factors concerned in the production of the anaemia apart from the presence or absence of achlorhydria were evenly distributed in both groups—for example, nutritional deficiency of iron and blood loss from the gastro-intestinal or genital tract.

The mean age and haemoglobin level in the achlorhydric group were 44.3 years and 8.1 g./100 ml., respectively, as compared with 46.8 years and 8.5 g./100 ml. in the group with acid in the gastric juice. Two of the achlorhydric group were males, while the other group contained seven males (Table I).

In all patients with a positive antibody test and a histamine-fast achlorhydria, serum vitamin-B<sub>12</sub> levels were estimated (Hutner *et al.*, 1956) and tests of vitamin-B<sub>12</sub> absorption and excretion were carried out with radio-cobalt (<sup>58</sup>Co)-labelled cyanocobalamin (Schilling, 1953). Where this latter test was abnormal the absorption of <sup>58</sup>Co-labelled vitamin B<sub>12</sub> taken together with intrinsic factor was also estimated.

### Results

**Iron-deficient Patients.**—Positive antibody tests were obtained in 11 out of 33 patients with histamine-fast achlorhydria, and in 2 out of 31 patients with acid in the gastric juice. The difference between these two groups is statistically significant ( $\chi^2=5.57$ ;  $P<0.02$ ).

**Control Subjects.**—Positive tests were obtained in 4 of the 64 controls, as compared with 13 positives among the 64 iron-

TABLE I.—Sex, Haemoglobin Levels, and Ages of Two Groups of Patients with Iron-deficiency Anaemia

	No.	Sex	Hb (g./100 ml.)		Age (Years)	
			Mean	S.D.	Mean	S.D.
Histamine-fast achlorhydria	33	31 F, 2 M	8.1	± 2.8	44.3	± 19.6
Acid in gastric juice	31	24 F, 7 M	8.5	± 2.4	46.8	± 22.4

\* From the University Departments of Medicine and Pathology, Western Infirmary, Glasgow.

deficient patients. There is thus a significantly increased incidence of antibodies in the iron-deficient group ( $\chi^2=4.34$ ;  $P<0.05$ ). This increase is attributable to the iron-deficient achlorhydric group, there being a highly significant difference in incidence when compared with the controls ( $\chi^2=10.25$ ;  $P<0.01$ ), while the incidence in the iron-deficient patients with acid in the gastric juice does not differ from the control group. Diagnex test meals were carried out in three of the four control subjects with positive tests; one of these had histamine-fast achlorhydria and two had acid in the gastric juice. The positive tests in the control subjects tended to occur in an older age-group (26, 70, 74, and 75 years; mean age 61.2 years) than in the anaemic patients (mean age 46.5 years).

**Vitamin-B<sub>12</sub> Studies.**—Of 11 achlorhydric patients with positive antibody tests three showed impaired absorption of radioactive vitamin B<sub>12</sub>; in two of these absorption was restored to normal by the administration of intrinsic factor. Serum-vitamin-B<sub>12</sub> levels were normal in all cases except one: this patient, whose Schilling test was suggestive of pernicious anaemia, had a serum vitamin-B<sub>12</sub> level of 66  $\mu\text{g./ml.}$  The haemoglobin, however, was restored to 14 g./100 ml. by iron therapy alone, and the bone-marrow was normoblastic (Table II).

TABLE II.—Schilling Tests and Serum Vitamin-B<sub>12</sub> Levels in 11 Iron-deficient Patients with a Positive Test for Fluorescent Antibodies and a Histamine-fast Achlorhydria

Subject	Age in Years	Schilling Test*		Serum Vitamin B <sub>12</sub> ( $\mu\text{g./ml.}$ )
		Without Intrinsic Factor (%)	With Intrinsic Factor (%)	
M.T. . .	31	14.1	—	196
A.McF. . .	41	23.2	—	392
C.W. . .	42	12	—	300
M.B. . .	43	16	—	248
A.A. . .	43	20.1	—	282
A.McL. . .	43	3.1	1.4	208
A.McI. . .	47	21	—	176
I.M. . .	48	16.2	—	256
M.P. . .	51	18.4	—	540
C.M. . .	56	1.2	15.1	66
M.P. . .	64	5.8	10	226

\* Urinary radioactivity expressed as percentage of dose of orally administered radioactivity.

## Discussion

These results confirm the finding of Markson and Moore (1962) that there is an increased incidence of gastric parietal-cell antibody in the sera of patients with iron-deficiency anaemia, and show, in addition, that the increased incidence occurs only in those patients who have a histamine-fast achlorhydria. It is already well established that a good correlation exists between the presence of a histamine-fast achlorhydria and the finding of a severe degree of chronic gastritis (Davidson and Markson, 1955; Bock *et al.*, 1963). Since chronic gastritis is the only outstanding lesion in the stomach in iron-deficiency anaemia, it appears likely that the autoantibody is associated with this pathological change. Indeed, since the completion of the present studies, Adams *et al.* (1964) have examined the sera of a large group of hospital patients for parietal-cell antibody. In 20 of these patients in whom the antibody was found gastric biopsy showed chronic gastritis of various grades of severity in every case.

Concerning the pathogenic significance of gastric autoantibody, it is now widely accepted that organ-specific autoantibodies in general are not of major pathogenic importance, and there is no reason to suppose that gastric autoantibody is an exception. It is possible, however, that chronic gastritis may result from an autoimmune mechanism, such as delayed hypersensitivity to gastric mucosal constituents, and that the autoantibody is merely a by-product of this. More evidence, including the investigation of the possible autoimmune nature of experimentally produced gastritis (Hennes *et al.*, 1962), is needed to elucidate this problem. If the immunological changes found predominantly in the achlorhydric patients of the present

series reflect an autoimmune process causative of atrophic gastritis, there would be reasonable grounds for suggesting that autoimmunity is a factor in the development of iron-deficiency anaemia; for Goldberg *et al.* (1963) have shown that in two well-matched groups of female patients with iron-deficiency anaemia, one having a histamine-fast achlorhydria and the other with acid in the gastric juice, the achlorhydric patients absorbed only one-third as much iron from an <sup>59</sup>Fe-labelled meal as the group with acid in the gastric juice.

A further question concerns the possible relation between iron-deficiency anaemia with achlorhydria and pernicious anaemia. The autoantibody to parietal cells detected in 86% of patients with pernicious anaemia (Taylor *et al.*, 1962) appears to be identical with that detected in patients with iron-deficiency anaemia with achlorhydria in the present investigation, although the incidence here is substantially lower. In only two of these patients was a Schilling test characteristic of pernicious anaemia found, and in only one of these was the serum vitamin-B<sub>12</sub> level low. A significantly reduced capacity to absorb vitamin B<sub>12</sub> may exist throughout life without the development of pernicious anaemia (McIntyre *et al.*, 1959). These findings, therefore, cannot be regarded as evidence that a positive antibody test necessarily predicts the future development of pernicious anaemia, but they do provide a link between the pathogenesis of the two conditions.

## Summary

The sera of 64 patients with iron-deficiency anaemia have been tested by an immunofluorescent technique for the presence of autoantibodies to the parietal cells of the gastric mucosa. Approximately one-half of these patients had a histamine-fast achlorhydria, the other half being able to secrete acid gastric juice.

The antibody was detected in 13 out of 64 iron-deficient patients (20.8%) and in 4 out of 64 normal control subjects (6.4%): the difference in incidence between these two groups is statistically significant ( $P<0.05$ ).

The antibody was present in 11 out of 33 anaemic (iron-deficient) patients with a histamine-fast achlorhydria, and in 2 out of 31 anaemic patients with acid gastric juice: the difference between the two groups is statistically significant ( $P<0.02$ ).

The relevance of these results to the pathogenesis of iron-deficiency anaemia and to the relation of iron-deficiency anaemia with pernicious anaemia is discussed.

We are indebted to Dr. R. A. Robb, of the department of mathematics, University of Glasgow, for the statistical analysis of our data, and to Dr. J. F. Adams, who carried out the serum-vitamin-B<sub>12</sub> estimations.

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