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PREDNISOLONE AND GASTRIC ATROPHY*

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SUMMARY

Eight patients with Addisonian pernicious anaemia were given 20 mg of prednisolone daily for up to 20 weeks. Improvement in absorption of vitamin B_{12} as judged by the Schilling test occurred in six cases and was pronounced in four, and there was increased secretion of gastric intrinsic factor in four cases. Gastric biopsies showed regeneration of specialized gastric glands in four cases; chief cells were demonstrated histochemically and parietal cells by an immunofluorescent procedure using serum containing parietal cell antibody. There was no correlative change in titre of serum antibody to gastric parietal cells or gastric intrinsic factor. The improved absorption of vitamin B_{12} was not maintained after prednisolone was stopped, indicating that the regenerated gastric mucosa reverted to the atrophic state. These effects of corticosteroids on gastric function in pernicious anaemia are in keeping with their known capacity to modify damage resulting from antigen–antibody interaction.

INTRODUCTION

Corticosteroids given to patients with Addisonian pernicious anaemia induce a reticulocyte response (Thorn *et al.*, 1950; Doig *et al.*, 1957), improved absorption of radioactive vitamin B_{12} (Frost & Goldwein, 1958; Gordin, 1959), and increased secretion of intrinsic factor (Kristensen & Friis, 1960). These effects can now be attributed to the capacity of prednisolone to induce glandular regeneration of the atrophic mucosa of pernicious anaemia (Jeffries, 1965; Ardeman & Chanarin, 1965; Jeffries, Todd & Sleisenger, 1966; Rødbro *et al.*, 1967). We present further data relating to the regenerative effect of prednisolone on the structure and function of the atrophic gastric mucosa of patients with pernicious anaemia. Histochemical and immunofluorescent staining established that the

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regenerated gastric glands contained both chief cells and parietal cells. This regenerative effect of prednisolone is in keeping with the concept that pernicious anaemia is a haematological state secondary to autoimmune gastritis.

MATERIALS AND METHODS

Eight patients with Addisonian pernicious anaemia were studied: seven were female and their ages were 48, 68, 72, 75, 75, 77, 80 and 85 years. All had advanced atrophic gastritis or gastric atrophy, histamine-fast achlorhydria and a grossly impaired capacity for absorption of vitamin B_{12}, corrected by intrinsic factor in the seven tested (Table 1). All had serum antibody to gastric parietal cells and six had antibody to gastric intrinsic factor. All had been treated with vitamin B_{12} for periods ranging from 1 month to 20 years. Four had lymphocytic thyroiditis diagnosed by thyroid biopsy, and two had diabetes mellitus.

Gastric suction biopsies (Wood *et al.*, 1949) were obtained at intervals before and after starting prednisolone. Two fragments were obtained. One fragment was processed for histological study including staining for pepsinogen granules of chief cells (Wood & Taft, 1958); the amount of specialized cells and lymphoid infiltration in the biopsy was graded as 0–3+. The other biopsy fragment was processed to demonstrate parietal cells by immunofluorescence, as follows: the patient's serum, containing antibody to parietal cells, was applied to a frozen section of the same patient's gastric mucosal biopsy, and binding of serum antibody to parietal cell cytoplasm was detected by anti-human globulin conjugated with fluorescein.

Gastric juice was collected by intermittent aspiration for 1 hr before and after the injection of 100 mg betazole hydrochloride ('histalog'). The pH was measured with universal indicator paper (Johnsons of Hendon Ltd, London). Gastric intrinsic factor was measured by the method of Ardeman & Chanarin (1963). Patients with pernicious anaemia secrete less than 100-ng units of intrinsic factor in the post-histamine hour, whereas most healthy subjects secrete over 1000-ng units/hr (Irvine, 1966). Vitamin B_{12} absorption was measured by the Schilling test (Schilling, 1953); a urinary excretion of 10% of the administered dose in 48 hr was our lower limit of normal. Antibodies to gastric parietal cells were detected by indirect immunofluorescence using rat stomach (De Boer, Nairn & Maxwell, 1965). Serum was titrated against parietal cell antigen by doubling dilutions in phosphate-buffered saline, pH 7·3; a difference in titre for a given patient was recorded if the end-points differed by two or more dilutions. Serum antibody to intrinsic factor was measured by the *in vitro* method of Gottlieb *et al.* (1965) and antibody to intrinsic factor in the gastric juice by a method similar to that of Fisher, Rees & Taylor (1966).

Prednisolone 20 mg daily was given in divided doses for up to 20 weeks with earlier withdrawal if normal vitamin B_{12} absorption occurred or if there were side effects or intolerance: observations as listed in Table 1 were made at intervals of 4–6 weeks.

RESULTS

The regenerative effect of prednisolone on the gastric mucosa in pernicious anaemia was assessed by improved absorption of vitamin B_{12}, increased secretion of gastric intrinsic factor and reappearance of both chief cells and parietal cells. Four patients (Cases 1–4)

showed a 'complete' response, one (Case 5) a partial response and one (Case 6) a transient response (Table 1).

TABLE 1. Effect of prednisolone in pernicious anaemia (functional and histological regeneration of the gastric mucosa was pronounced in Cases 1–4, partial in Case 5 and transient in Case 6)

	Case No.							
	1	2	3	4	5	6	7	8
Age and sex	75 F	85 F	77 F	68 F	80 F	48 F	75 F	72 M
Duration of disease (years)	7	8	8	15	16	0·1	18	14
Prednisolone								
Duration (weeks)	5	20	8	6	16	12	9	20
Total dose (g)	0·70	2·80	1·12	0·63	2·24	1·68	1·26	2·80
Schilling test (% oral dose excreted)								
Before prednisolone	3	3	0	4	1	5	0	1
(Before prednisolone with hog I.F.)	(16)	n.t.	(11)	(11)	(12)	(14)	(6)	(14)
During prednisolone	14	14	10	12	5	9*, 4	3	2
After prednisolone	1	2	n.t.	1	1	n.t.	n.t.	2
Gastric intrinsic factor (1 hr post-histalog)								
Before prednisolone	0	0	30	150	190	70	130	n.t.
During prednisolone	650	100	480	400	30	110*, 40	350	n.t.
pH of gastric juice								
Before prednisolone	7·5	7·5	8·0	7·5	8·0	7·0	7·0	7·0
During prednisolone	7·0	8·0	8·0	5·0	8·0	7·0	7·0	7·0
Gastric histology (0–4+ scale)								
Specialized cells								
Before prednisolone	0	0	0	+	0	+	0	0
During prednisolone	++	++	n.t.	++	+	0	0	0
Lymphoid cells								
Before prednisolone	+++	+++	+	+++	+++	++	++	+
During prednisolone	++	++	n.t.	+	++	+	++	+
Serum antibody titre (parietal cell)								
Before prednisolone	16	8	4	4	2	4	4	16
During prednisolone	8	0	4	4	8	4	4	16
Intrinsic factor antibody (ng units/ml)								
Before prednisolone	13	0	8	0	66	154	7	60
During prednisolone	0	0	0	0	40	166*, 205	6	50

I.F., Intrinsic factor; n.t., not tested.

* Values after 6 weeks of prednisolone.

Improvement of vitamin B_{12} absorption was unequivocal in four patients, partial in one and transient in one (Case 6) in whom the results of serial Schilling tests were 5, 9 and 4% of a 1-μg dose of vitamin B_{12}. After stopping prednisolone, three of the four patients

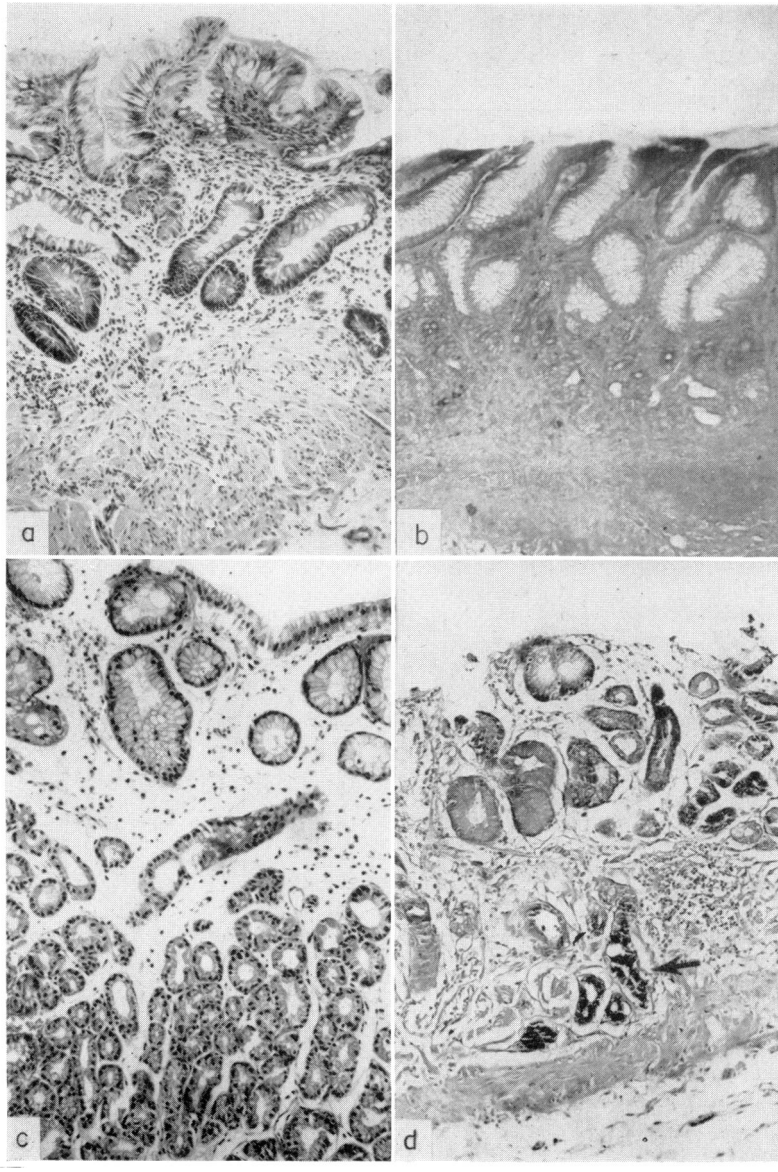

FIG. 1. Gastric biopsies of a patient with pernicious anaemia (Case 1) before prednisolone (a and b) and during prednisolone (c and d). (a) Gastric mucosa showing atrophy and collections of lymphoid cells. H & E, ×100. (b) Gastric mucosa showing absence of chief cells by use of stain for pepsinogen granules (cf. d). ×100. (c) Gastric mucosa showing a number of regenerated gastric glands. H & E, ×100. (d) Gastric mucosa showing presence of chief cells (arrow) by use of stain for pepsinogen granules. ×100.

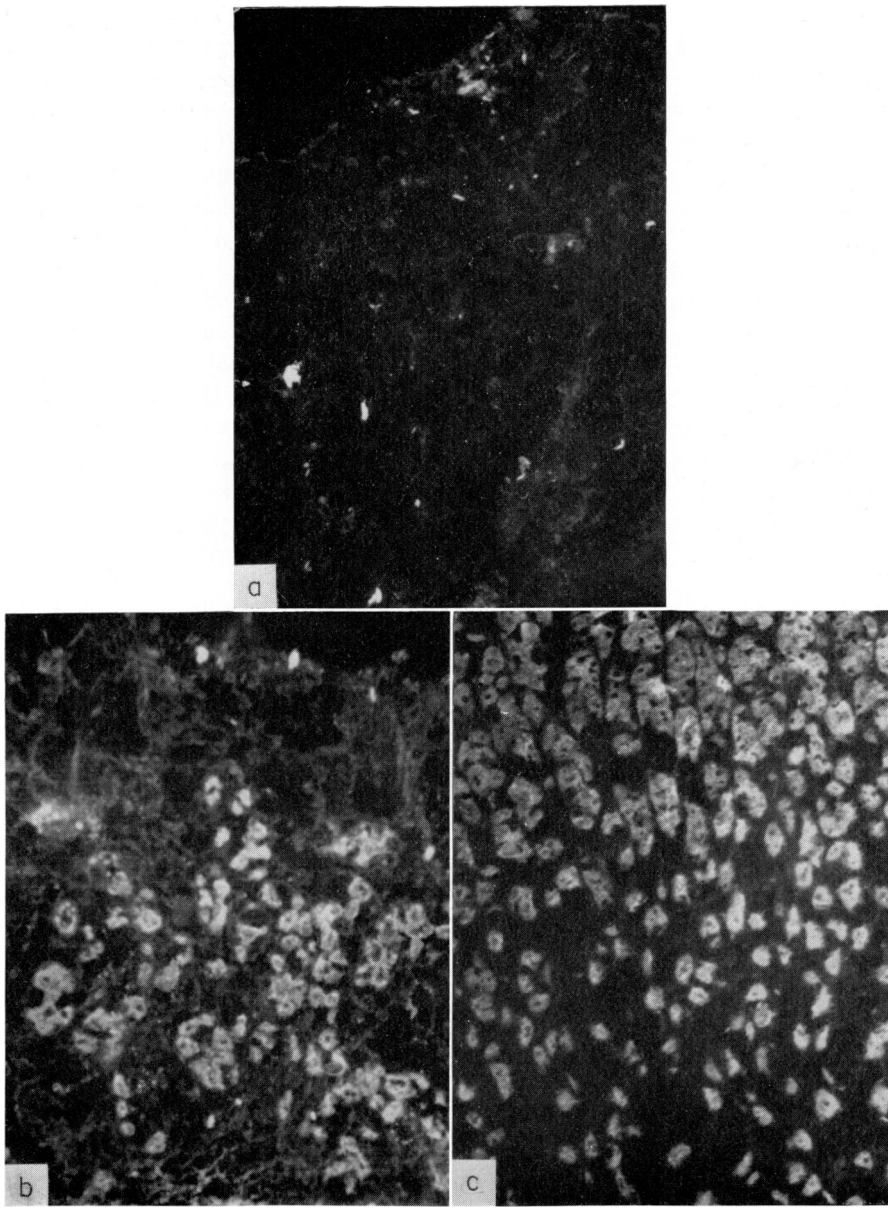

FIG. 2. Immunofluorescence tests on frozen sections of gastric biopsies illustrating regeneration of parietal cells. The frozen section of the gastric biopsy was layered with the serum of that patient (Case 2), washed, and treated with a fluorescein-conjugated antigamma globulin serum. The presence of parietal cells was indicated by specific fluorescence. (a) Biopsy before prednisolone was given showing absence of parietal cells. ×400. (b) Biopsy 6 weeks after starting prednisolone showing numbers of regenerated parietal cells. ×400. (c) A biopsy of a normal gastric mucosa, used as a positive control, showing reaction of patient's serum (Case 2) with parietal cells. ×400.

who had developed normal vitamin B_{12} absorption reverted to pre-treatment levels 6, 7 and 12 weeks later; the other was not tested. The gastric juice remained alkaline in seven patients, but in one (Case 4) there was a change from pH 7 to pH 5. Before prednisolone the secretion of gastric intrinsic factor was low, below 200-ng units/hr, in all patients. After prednisolone, secretion of intrinsic factor increased markedly in four patients (Cases 1, 3, 4 and 7) and to a lesser extent in two patients (Cases 2 and 6). Incomplete aspiration of viscid gastric juice could have accounted for the apparent fall in secretion in one patient (Case 5), and a rise inconsistent with the improved vitamin B_{12} absorption in two others (Cases 2 and 6).

The histological appearances of forty-four pre-treatment biopsies from the eight patients, obtained up to 16 years previously, were remarkably constant. All showed mucosal atrophy. Intestinal metaplasia was present in all biopsies from six patients; a few parietal cells were present in the biopsies of two patients (Cases 4 and 6). Lymphoid infiltration was scored as 3+ in four, 2+ in two and 1+ in two patients. We examined thirty-two biopsies from seven patients whilst they were taking prednisolone. Four patients showed regeneration of gastric glands containing specialized cells and five showed reduction of lymphoid infiltration (Table 1, Fig. 1a and b). Chief cells were demonstrated histochemically (Fig. 1c and d) and an immunofluorescence test, using the specific reaction of the patient's serum with their gastric parietal cells, demonstrated parietal cells in the regenerated mucosa (Fig. 2a–c). All four patients (Cases 1, 2, 4 and 5) in whom gastric biopsy showed regeneration of specialized cells had improved absorption of vitamin B_{12}.

Antibody to gastric parietal cells was present in low titre (not >16) in the eight patients. After prednisolone the titre remained unchanged in six, fell in one whose response was complete and rose in another whose response was partial; there was no change in titre over 3 months after withdrawal of prednisolone. Antibody to gastric intrinsic factor was present in low titre in three and in high titre in three patients. After prednisolone the titre fell in two patients (Cases 1 and 3) whose response was complete, in one (Case 5) who showed partial response and in one (Case 8) who showed no response, and rose in one patient (Case 6) whose absorption of vitamin B_{12} improved initially but later deteriorated while she was still taking prednisolone. Antibody to gastric intrinsic factor was not detected in the gastric juice of any patient. Free intrinsic factor was detected in the gastric juice of six of the seven patients tested.

Some patients had temporary symptoms which could be attributed to the effects of prednisolone: six had facial 'mooning', three symptoms of anxiety, one paranoid delusions, two indigestion and upper abdominal pain, one became hypertensive, one had glycosuria and one transient hemiparesis. Prednisolone was withdrawn from six patients before the end of the planned 20 weeks. Two patients aged 77 and 85 years had a hemiparesis, which was transient in one and fatal in the other, within 2 months of withdrawal of prednisolone, but the relationship of hemiparesis to prednisolone was uncertain.

DISCUSSION

We demonstrated improved vitamin B_{12} absorption in six of eight patients with pernicious anaemia who were given prednisolone for up to 20 weeks. This improvement was accompanied by increased secretion of gastric intrinsic factor and regeneration of both chief cells and parietal cells, said to be the source of intrinsic factor (Hoedemaeker *et al.*, 1964). These results are in accord with those of Jeffries and colleagues (Jeffries, 1965; Jeffries

et al., 1966), Ardeman & Chanarin (1965) and Rødbro *et al.* (1967). We could not in our cases show any secretion of gastric acid during the giving of prednisolone. The increased absorption of vitamin B_{12} with the giving of prednisolone was clearly attributable to increased secretion of gastric intrinsic factor in three of our patients but we could not demonstrate this in the other three patients, perhaps because of incomplete aspiration of viscid gastric juice. The gastric mucosa presumably returned to the atrophic state soon after withdrawal of prednisolone, since vitamin B_{12} absorption then reverted to pre-treatment levels.

The histological appearance of the atrophic gastric mucosa of pernicious anaemia would suggest a static end-stage of an antecedent destructive process. However, Croft, Pollock & Coghill (1966) showed that in pernicious anaemia there is a normal rate of turnover of gastric mucosal cells, indicating that the gastric mucosa has the capacity for regeneration. We presume that corticosteroids counteract an immunodestructive process, so permitting mucosal regeneration to occur. Jeffries *et al.* (1966) mentioned that prednisolone could have a 'non-specific' stimulating effect on the gastric mucosa but, as shown by Ardeman & Chanarin (1965), gastric mucosal atrophy associated with post-gastrectomy gastritis was not influenced by prednisolone.

Pernicious anaemia has been known to fulfil certain of our 'markers' for an autoimmune disease (Mackay & Burnet, 1963), these including a high incidence of autoantibodies to gastric and thyroid components, lymphoid infiltration of the target tissue, and coexistence with other autoimmune disorders, chiefly Hashimoto's thyroiditis. To these might now be added responsiveness of the gastric lesion to corticosteroid drugs. Our inability to relate the regenerative effect of prednisolone to changes in titre of antibodies to parietal cells or intrinsic factor was not unexpected, since one effect of corticosteroids in immunopathological processes is to lessen or prevent damage resulting from antigen–antibody interaction. A point not often considered in gastric autoimmunity relates to atrophy of the chief cells and the smooth muscle of the stomach. Although autoantibody to chief cells has not been demonstrated, these also regenerate under the influence of prednisolone.

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