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Serum from patients with pernicious anaemia blocks gastrin stimulation of acid secretion by parietal cells

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SUMMARY

We examined 51 sera from patients with pernicious anaemia for their capacity to block maximal gastrin stimulation of acid secretion by isolated rodent gastric parietal cells. ^{14}C-aminopyrine accumulation was used as the index of acid secretion *in vitro*. Sera from patients with pernicious anaemia gave significantly ($P < 0{\cdot}005$) more block of maximal gastrin stimulation of acid secretion ($61{\cdot}7 \pm 37{\cdot}8\%$) than sera from 10 patients with systemic lupus erythematosus ($19{\cdot}6 \pm 17{\cdot}7\%$), 10 with scleroderma ($34{\cdot}2 \pm 22{\cdot}3\%$), five with rheumatoid arthritis ($22{\cdot}4 \pm 15{\cdot}6\%$) or 30 from healthy persons ($27{\cdot}4 \pm 12{\cdot}8\%$). Maximal histamine stimulation of acid secretion was not inhibited. The blocking factor was present in serum IgG fractions, and serum and IgG fractions gave parallel dose–response and dilution curves. The serum block was abolished by absorption with gastric mucosal cells and correlated with the presence of parietal cell surface autoantibody. We conclude that serum immunoglobulin in pernicious anaemia can block gastrin stimulation of acid secretion and suggest that this block may be mediated by competition with gastrin for surface receptors on parietal cells.

Keywords parietal cell blocking autoantibody gastrin ^{14}C-aminopyrine uptake

INTRODUCTION

Chronic atrophic gastritis, characterized by depletion of parietal cells from the gastric mucosa, is the principal pathological lesion of pernicious anaemia (Strickland & Mackay, 1973). We, and others, using indirect immunofluorescence (Masala *et al.*, 1980; de Aizpurua, Toh & Ungar, 1983a) and flow microfluorometry (de Aizpurua *et al.*, 1983b) have demonstrated the presence of autoantibodies reactive with the surface membranes of isolated parietal cells. Subsequently, we showed that these surface reactive autoantibodies may be cytotoxic to parietal cells *in vitro*, and suggested that these antibodies may contribute to the loss of such cells from the gastric mucosa of patients with pernicious anaemia (de Aizpurua, Ungar & Toh, 1983c).

Gastrin is a gastrointestinal hormone, produced by antral G cells, which stimulates HCl secretion by gastric parietal cells and which also has a trophic action on the gastric mucosa (Johnson, 1981). The physiological actions of gastrin are probably mediated by the interaction of the hormone with cell surface receptors on parietal cells (Soll *et al.*, 1984). The accessibility of cell surface receptors for hormones and neurotransmitters also makes these receptors accessible to autoantibodies which may compete with the natural ligand for access to the receptor site (Lennon & Carnegie, 1971; Carnegie & Mackay, 1975). The hypothesis is supported by the findings of

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autoantibodies to thyrotropin receptors in Graves' disease (Adams & Purves, 1965), to acetylcholine receptors in myasthenia gravis (Nittag *et al.*, 1976), to insulin receptors in a rare form of insulin resistant diabetes mellitus (Flier *et al.*, 1975) and to β-adrenoceptors in allergic asthma (Venter, Fraser & Harrison, 1980).

In the present study, we set out to test the hypothesis that autoantibody to gastrin receptors may be present in pernicious anaemia. For this purpose, we examined sera and serum IgG fractions from patients with pernicious anaemia for their capacity to block gastrin stimulation of acid secretion by isolated rodent gastric parietal cells. We selected ^{14}C-aminopyrine accumulation in parietal cells as an indirect index of HCl secretion because of its simplicity, sensitivity and reproducibility, and because of its good correlation with the results of oxygen consumption studies, another index of acid secretion (Soll, 1980; Chew & Hersey, 1982). Introduced by Berglindgh, Helander & Obrink (1976) the bioassay is based on the principle that ^{14}C-aminopyrine becomes trapped in the acid spaces of stimulated parietal cells when the pH falls below its pKa of 5·0 because of conversion to an ionized form which diffuses poorly across plasma membranes. The sensitivity of the assay can be increased by the addition of dithiothreitol (Chew & Hersey, 1982) which acts by an unknown mechanism.

MATERIALS AND METHODS

Sera. Sera from 51 Caucasian patients with pernicious anaemia attending the Royal Melbourne Hospital were examined for their capacity to block gastrin stimulated acid secretion by parietal cells. The patients, comprising 35 women and 16 men, aged between 30 and 89 years (mean age 64 years), formed part of a larger patient series whose sera have previously been examined for parietal cell, intrinsic factor and cytotoxic autoantibodies (de Aizpurua *et al.*, 1983a, 1983b, 1983c). Control serum samples were drawn from 10 patients with systemic lupus erythematosus, 10 with scleroderma, five with rheumatoid arthritis and 30 from healthy persons. Seven of the patients with systemic lupus erythematosus were on maintenance corticosteroid treatment.

Sera from 10 patients with pernicious anaemia which gave > 55% block of gastrin stimulated acid secretion by parietal cells were selected for preparation of IgG fractions using a method as previously described (de Aizpurua *et al.*, 1983c). IgG fractions were also similarly prepared from five sera from patients with systemic lupus erythematosus, five from rheumatoid arthritis, five from scleroderma and 10 from healthy persons.

Gastric parietal cell enriched cell suspensions. For each bioassay, stomachs, freshly obtained from three male Sprague-Dawley rats, fed and watered *ad libitum*, were opened, gently scraped with a glass slide to remove surface mucus and everted. Gastric parietal cell enriched cell suspensions were dissociated from these cleaned, everted, stomachs by collagenase using a method described previously (de Aizpurua *et al.*, 1983c). The method was modified to improve cell yield by the addition of 4 mM calcium chloride to the collagenase dissociation steps and to improve cell viability by suspending the cells in modified Eagle's medium containing 10% fetal calf serum. The final cell suspension, containing 70–90% (mean 80%) parietal cells with a viability of > 90% as assessed by trypan blue dye exclusion, was adjusted to a concentration of 3×10^6 cells/ml.

^{14}C-aminopyrine accumulation bioassay. The method for the ^{14}C-aminopyrine bioassay as an index of gastric acid secretory activity has been described in detail elsewhere (Soll, 1980, Chew & Hersey, 1982). For our experiments, the method was modified as follows. One millilitre aliquots of parietal cell enriched cell suspensions containing 3×10^6 cells/ml in modified Eagle's medium containing 10% fetal calf serum were placed in 1·5 ml conical polypropylene tubes and incubated for 20 min at room temperature with sera (1:20 final dilution) or IgG fractions from patients with pernicious anaemia. The cells were washed twice in the serum supplemented modified Eagle's medium by centrifugation at $250g_{max}$ for 5 min and resuspended in 1 ml of the same medium containing 3 mM ^{14}C-aminopyrine (Amersham, Arlington Heights, Illinois, USA), specific activity 114 mCi/mmol, 10^{-7}M synthetic human 15-Leu-gastrin-17 (Fluka, AG, Buchs, Switzerland) and 0·5 mM dithiothreitol (Sigma, St Louis, Missouri, USA). Preliminary experiments had established that 10^{-7}M 15-Leu-gastrin-17 gave maximum ^{14}C-aminopyrine accumulation in parietal cells.

These resuspended cells in medium containing ^{14}C-aminopyrine, gastrin and dithiothreitol, were incubated with regular shaking (120 cycles per minute) for 45 min at 37°C in an atmosphere containing 5% CO_2/95% air. Preliminary experiments had established that these conditions were optimal for block of maximal gastrin stimulated ^{14}C-aminopyrine accumulation in parietal cells by sera from patients with pernicious anaemia. At the end of the 45 min incubation period, duplicate 0·5 ml aliquots of each sample were washed in 1 ml Hank's balanced salt solution by centrifugation at 1,000g_{max} for 1 min. The supernatants were aspirated and the cell pellets digested in 500 μl of Protosol (New England Nuclear, Boston, Massachusetts, USA) for 20 min at 50°C. The digested samples, in aqueous scintillation cocktail, were counted in a β-counter (Packard, Downers Grove, Illinois). Included in each run were a positive control of cells exposed to ^{14}C-aminopyrine, gastrin and dithiothretol with omission of the serum pre-incubation step, and a negative control of cells exposed to ^{14}C-aminopyrine alone.

The results of the ^{14}C-aminopyrine accumulation bioassay was expressed as an accumulation ratio (Soll, 1980, Chew & Hersey, 1982) calculated as follows:

$$\text{accumulation ratio, AP} = \frac{\text{ct/min}_p/\text{total parietal cell volume}}{\text{ct/min}_m}$$

where ct/min_p is the counts per minute in the cell pellet and ct/min_m is the counts per minute in the incubation medium. The total parietal cell volume was calculated as the product of the number of cells in the pellet, the ratio of parietal cells to total cells and the mean volume of each parietal cell. Taking 22 μm as the average diameter of each parietal cell (average of the diameters of 100 parietal cells), the mean volume of each parietal cell was determined to be $3{\cdot}3 \times 10^3$ μm.

The capacity of sera to block maximal gastrin stimulated ^{14}C-aminopyrine accumulation was expressed as a percentage of the maximal accumulation calculated as follows:

$$\%\ \text{specific block} = \frac{(AR_G - AR_B) - (AR_{G+S} - AR_B) \times 100}{AR_G - AR_B}$$

where AR_G is the accumulation ratio with maximal gastrin stimulation, AR_{G+S} the accumulation ratio for cells incubated with serum before exposure to gastrin and AR_B is the accumulation ratio for basal, unstimulated cells.

Experiments were also carried out with sera and IgG fractions from patients with pernicious anaemia, in the presence of different gastrin concentrations and with increasing dilutions of sera and IgG fractions.

Specificity studies. Specificity of the block of gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells by sera and IgG fractions from patients with pernicious anaemia was determined as follows.

Firstly, specificity for pernicious anaemia was ascertained by tests with sera and IgG fractions from patients with systemic lupus erythematosus, scleroderma, rheumatoid arthritis and normal controls for their capacity to block maximal gastrin stimulation of ^{14}C-aminopyrine accumulation by parietal cells.

Secondly, specificity of the block for gastrin was determined by parallel experiments with maximal histamine stimulation of ^{14}C-aminopyrine accumulation by parietal cells. Preliminary experiments had established that maximal ^{14}C-aminopyrine accumulation was obtained with 10^{-5}M histamine (Calbiochem, La Jolla, California, USA.) and that the accumulation could be specifically blocked by 10^{-5}M of the histamine antagonist cimetidine (Sigma).

Thirdly, specificity of the block for parietal cells was sought by experiments with 10 blocking sera which had been absorbed (de Aizpurua *et al.*, 1983b) with parietal cell enriched gastric mucosal cells, liver cells or kidney cells.

Correlation with parietal cell, intrinsic factor and cytotoxic autoantibodies. All 51 sera had previously been tested for parietal cell, intrinsic factor and cytotoxic autoantibodies (de Aizpurua *et al.*, 1983a, 1983b, 1983c). The presence of serum blocking activity was correlated with the presence of these autoantibodies.

Statistical methods. A two sample, two tailed Student's *t*-test and two-tailed Wilcoxan rank sum test was used for statistical analysis of results.

Table 1. Inhibition of gastrin stimulated ^{14}C-aminopyrine accumulation in parietal cells by sera from patients with pernicious anaemia

	Accumulation ratio (mean ± s.d.)	
	10^{-7}M gastrin	10^{-5}M histamine
Maximally stimulated cells	25·2 ± 3·3 (n = 30)	45·7 ± 3·8 (n = 20)
Basal, unstimulated cells*	12·2 ± 1·4	10·4 ± 2·1
Sera from patients with pernicious anaemia		
No absorption	12·8 ± 2·1 (n = 10)	46·0 ± 2·1 (n = 51)
Absorption with gastric mucosal cells	21·2 ± 1·9	Not tested
Absorption with liver cells	14·5 ± 1·7	Not tested
Absorption with kidney cells	14·6 ± 2·1	Not tested
Sera from normal controls (n = 30)	21·0 ± 2·9	45·2 ± 2·0

*The accumulation ratios in basal, unstimulated cells were not inhibited by test or control sera.

RESULTS

Serum block of ^{14}C-aminopyrine accumulation

Fig. 1a shows that the block of maximal gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells is significantly ($P < 0.005$) greater with sera from 51 patients with pernicious anaemia (61·7 ± 37·8%) than with sera from 10 patients with systemic lupus erythematosus (19·6 ± 17·7%), 10 with scleroderma (34·2 ± 22·3%), five with rheumatoid arthritis (22·4 ± 15·6%) or 30 from healthy persons (27·4 ± 12·8%). In contrast, there is no significant inhibition ($P > 0.05$) of maximal

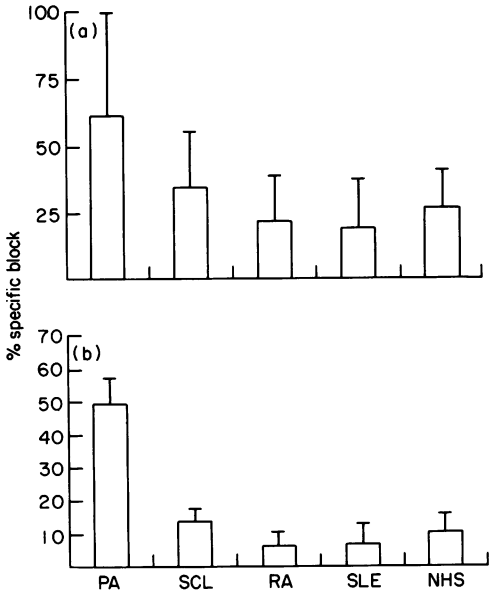

Fig. 1. Block of 10^{-7}M gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells by (a) serum or (b) serum IgG fractions from patients with pernicious anaemia (PA), scleroderma (SCL), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or from healthy persons (NHS). Histograms represent mean + s.d.

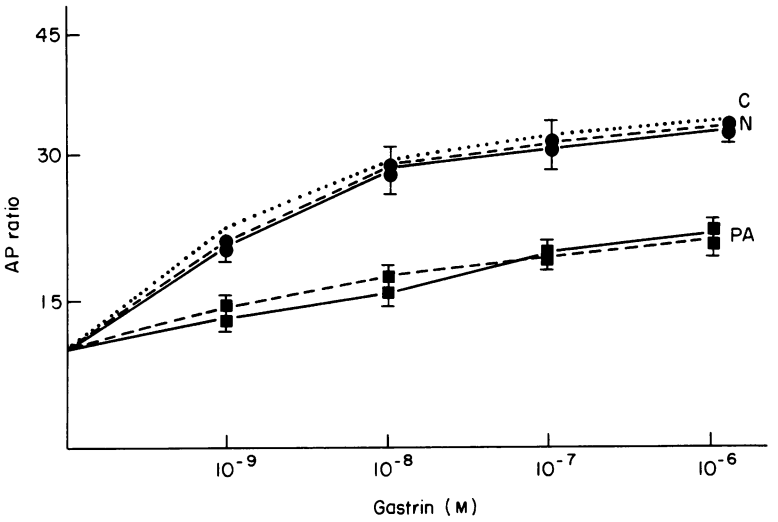

Fig. 2. Inhibition of stimulation of ^{14}C-aminopyrine accumulation in parietal cells by increasing concentration of gastrin, expressed as an aminopyrine accumulation ratio (AP ratio), by serum (——) or serum IgG fraction (– – –) from a patient with pernicious anaemia (PA). Control cells not exposed to serum (C) or exposed to serum (——) or serum IgG fraction (– – –) from a healthy person (N) shown for comparison. Points and bars represent mean ± s.d.

histamine stimulation of ^{14}C-aminopyrine accumulation in parietal cells with sera from patients with pernicious anaemia or with sera from healthy persons (Table 1). Table 1 also shows that inhibition of maximal gastrin stimulated ^{14}C-aminopyrine accumulation by 10 sera from patients with pernicious anaemia is abolished by prior absorption of the sera with parietal cell enriched gastric mucosal cells but not by absorption with liver cells or kidney cells. The block of gastrin stimulated ^{14}C-aminopyrine accumulation was not related to the patient's age, disease duration or ABO blood groups antigens. Fifty-five per cent of sera from patients with pernicious anaemia gave a block > 53% (mean + 2 s.d. of block obtained with control sera).

Fig 1b shows that serum IgG fractions from 10 patients with pernicious anaemia gave significantly ($P < 0{\cdot}001$) more block (49·2 ± 8·4%) than the serum IgG fractions from five patients

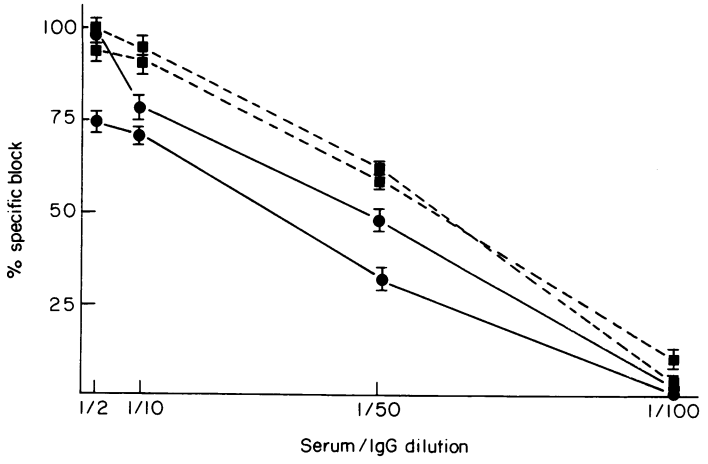

Fig. 3. The effect of increasing dilutions of serum (——) or serum IgG fractions (– – –) from two patients with pernicious anaemia on the block of 10^{-7}M gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells. Points and bars represent mean ± s.d.

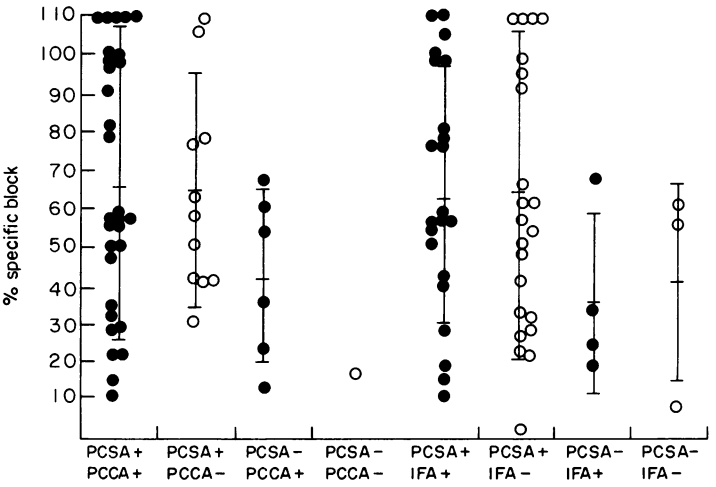

Fig. 4. Correlation of serum block of 10^{-7}M gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells with the presence or absence of parietal cell surface antibody (PCSA), parietal cell cytoplasmic antibody (PCCA) and intrinsic factor antibody (IFA). Bars represent mean ± s.d.

with systemic lupus erythematosus (7·1 ± 5·5%), five with scleroderma (13·8 ± 3·3%), five with rheumatoid arthritis (5·8 ± 6·4%) and 10 from healthy persons (10·2 ± 5·5%). All control sera as well as IgG fractions showed some degree of 'non-specific' block of maximum gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells (Fig. 1).

The inhibition of gastrin stimulated ^{14}C-aminopyrine accumulation by serum or IgG fractions seen with different concentrations of gastrin (Fig. 2) and with increasing dilutions of serum or IgG fractions (Fig. 3) parallel one another.

Correlation of serum block with parietal cell, intrinsic factor and cytotoxic autoantibodies

The block of maximal gastrin stimulated ^{14}C-aminopyrine accumulation by sera from patients with

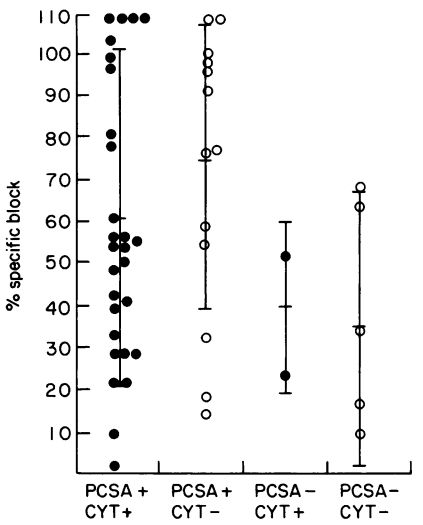

Fig. 5. Correlation of serum block of 10^{-7}M gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells with the presence or absence of parietal cell surface antibody (PCSA) and parietal cell cytotoxic antibody (CYT). Bars represent mean ± s.d.

pernicious anaemia correlated with the presence of parietal cell surface autoantibody (Figs 4 & 5). Thus, the block obtained with 44 sera from patients with pernicious anaemia that were positive for parietal cell surface antibody ($66{\cdot}5 \pm 34{\cdot}8\%$) was significantly ($P < 0{\cdot}005$) greater than that obtained with seven sera that were negative for parietal cell surface antibody ($38{\cdot}8 \pm 22{\cdot}0\%$). The block obtained with the sera which were negative for parietal cell surface autoantibody was not significantly different ($P > 0{\cdot}05$) from that of control sera. In contrast, the serum block did not correlate with the presence or absence of parietal cell cytoplasmic antibody, intrinsic factor antibody (Fig. 4) or cytotoxic antibody (Fig. 5).

DISCUSSION

The possibility that parietal cell surface reactive autoantibodies (de Aizpurua *et al.*, 1983a, 1983b) may compete with gastrin for gastrin receptors on parietal cells with block of hormone action is supported by the earlier observation that gammaglobulins from 13 patients with pernicious anaemia blocked spontaneous acid secretion by the living gastric mucosa of bull frogs and that gammaglobulins from two patients blocked gastrin stimulation of carbonic anhydrase activity of strips of the fundus from guinea pig stomachs (Loveridge *et al.*, 1980). The recent observation that elevated serum gastrin levels are found in normal subjects with parietal cell autoantibodies and that this association is independent of gastric pH is also consistent with this suggestion (Bins *et al.*, 1983).

Our results show that sera from 51 patients with pernicious anaemia blocked maximal gastrin stimulation of ^{14}C-aminopyrine accumulation in parietal cells and that this block was significantly ($P < 0{\cdot}005$) greater than that obtained with sera from 55 controls. The serum block seems specific for gastrin since no significant inhibition of maximal histamine stimulation of ^{14}C-aminopyrine accumulation was obtained with sera from patients with pernicious anaemia or with sera from controls. All sera and serum IgG fractions gave some 'non-specific' block of maximum gastrin stimulation of ^{14}C-aminopyrine accumulation. In Graves' disease, serum IgG fractions have also been shown to give 'non-specific' block of thyrotropin binding to thyroid tissue (Beall *et al.*, 1978). The cause of 'non-specific' block is not known.

The serum blocking factor was present in the IgG fractions of serum immunoglobulins and in both dose–response and dilution experiments, the curves for block by serum and IgG fractions paralleled one another. The block was specific for parietal cells since it can be abolished by absorption with parietal cells enriched gastric mucosal cells but not by absorption with liver cells or kidney cells.

The serum blocking factor correlates with the presence of parietal cell surface autoantibody but not with parietal cell cytoplasmic or intrinsic factor antibody suggesting that the serum block is probably mediated by parietal cell surface autoantibody and not by the other types of autoantibodies. The lack of correlation between the blocking factor and parietal cell cytotoxic antibody, which itself correlates with the presence of parietal cell surface antibody (de Aizpurua *et al.*, 1983b), suggests that parietal cell surface autoantibodies are probably heterogeneous and includes those with blocking, cytotoxic or both blocking and cytotoxic activities. The concurrent presence of blocking and cytotoxic autoantibodies has also been reported in experimental models of myasthenia gravis and probably also exists in the corresponding human disease (Lennon, 1979).

The demonstration that 55% of sera from patients with pernicious anaemia have blocking activity on rodent gastric parietal cells suggests that the blocking antibody may be recognizing antigenic determinants on gastrin receptors which may be shared in common between rodents and humans. As is the case with autoantibody to thyrotropin receptors in Graves' disease (Adams & Kennedy, 1971) it is possible that autoantibodies to antigenic determinants on gastrin receptors which are unique to the human species exist, and that the percentage positivity of serum blocking activity may be higher with the use of human gastric parietal cells.

Whether serum from patients with pernicious anaemia would also block the trophic action of gastrin is not known. However, as the trophic receptor for gastrin in dividing gastric mucous neck cells, is probably identical to the gastrin receptor on parietal cells (Johnson, 1981), it is probable that the serum blocking antibody would also block the trophic effects of gastrin. The pathogenetic

significance of the blocking antibody may rest mainly on its capacity to block cell renewal following parietal cell destruction by cytotoxic autoantibody. The demonstration that, in pernicious anaemia, parietal cells may be generated by corticosteroid treatment (Jeffries, 1965) but do not do so in the absence of steroid treatment, despite high circulating gastrin levels (McGuigan & Trudeau, 1970) which should provide a maximal trophic stimulus, is consistent with the suggestion that cell renewal may be impaired by a circulating blocking autoantibody. The report (Kaye, Whorwell & Wright, 1983) that most lymphocytes infiltrating the gastric mucosa of patients with pernicious anaemia are non-T cells and are probably B cells raises the possibility that local production of blocking and cytotoxic autoantibodies may also have a role in parietal cell loss from the gastric mucosa.

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