AUTOIMMUNE PHENOMENA IN PERNICIOUS ANAEMIA: GASTRIC ANTIBODIES

BY

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The presence of an inhibitor of intrinsic factor activity was demonstrated in the sera of some patients with pernicious anaemia who had never been treated with hog intrinsic factor (Taylor, 1959b; Schwartz, 1960). It was proposed that this inhibitor, which was precipitated with the serum globulin fraction, might be an auto-antibody. This supported the earlier suggestions, based upon the histological appearances of the gastric mucosa, that pernicious anaemia might have an autoimmune component (Taylor, 1959a). Initial attempts to determine the nature of the serum factor by precipitation and tanned red-cell techniques were inconclusive (Taylor and Boyden, quoted by Schwartz, 1960; Abels *et al.*, 1962).

There is a known clinical association between pernicious anaemia and myxoedema (Tudhope and Wilson, 1960, 1962), a disease in which autoimmunity has been strongly implicated (Doniach and Roitt, 1957). Further, post-mortem examination of the thyroids of patients with pernicious anaemia has revealed a high incidence of thyroiditis (Bastenie, 1937; Williams and Doniach, 1962). These associations led us to study the auto-antibody pattern in pernicious anaemia with particular reference to specific gastric and thyroid auto-antibodies. In the present paper we report our studies on the nature and incidence of auto-antibodies to components of the gastric mucosa. The clinical and serological overlap of pernicious anaemia with thyroiditis will be presented in another paper (Doniach, Roitt, and Taylor, to be published).

Materials and Methods

Sera were obtained from 100 patients with pernicious anaemia attending the anaemia clinic of the Nuffield Department of Clinical Medicine, Oxford, and from 43 patients at the Middlesex Hospital. The Oxford patients were unselected, of either sex, and of ages ranging from 27 to 91 years. All had been fully investigated and a diagnosis of pernicious anaemia made on the criteria of typical peripheral blood and marrow findings, failure of gastric acid production after histamine stimulation, barium-meal examination, vitamin-B_{12} activity in serum, tests of absorption of labelled vitamin B_{12}, and gastric biopsy. In patients in whom the diagnosis was still in doubt, tests of intestinal absorption had been done to exclude disease of the small intestine. All were receiving adequate treatment with vitamin B_{12} by injection and none had received any treatment with heterologous intrinsic factor preparations.

The Middlesex Hospital series included some patients referred for thyroid antibody tests because of overt or suspected thyroid disease; they are therefore partly selected.

Control sera were obtained from subjects in apparent good health, matched for age and sex with the Oxford series. Sera were stored at $-20°$ C. for up to two years.

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Preparation of Antigens

1. Hog Intrinsic-Factor–Vitamin-B_{12} Complex (I.F.-B_{12}).—^{58}Co-B_{12} (5 μg.; spec. activity 10 μc./μg.) was added to a preparation of hog intrinsic factor (10 mg.; Lederle) dissolved in 0.15 M buffered saline and dialysed for 48 hours.

2. Human I.F.-B_{12} Complex.—^{58}Co-B_{12} (800 mμg.; spec. activity 10 μc./μg.) was added to lyophilized human gastric juice (13 mg. of protein) dissolved in 1 ml. of buffered saline, and the I.F.-B_{12} complex was purified by starch gel electrophoresis as described by Jeffries *et al.* (1962). The gel containing the peak of anodal radioactivity was frozen and thawed and the fluid expressed under pressure in a syringe. This was stored in the frozen state.

3. Gastric Mucosa Homogenate.—The mucosa was stripped from the healthy fundal portion of fresh human stomach removed at operation from blood group O patients suffering from duodenal or gastric ulcer, or from carcinoma of the stomach. The tissue was washed in buffered saline, finely scissored, and disrupted in an all-glass homogenizer with 4 volumes of 10% sucrose. After filtering through muslin, mucus, cell debris, and nuclei were removed by centrifuging at 2,000 r.p.m. (800 *g*) for 10 minutes. The supernatant was then spun at 40,000 r.p.m. in a Spinco model L for 50 minutes and the deposit was resuspended in borate buffer to the original volume of homogenate and used as antigen for complement-fixation tests.

The same procedure was used for the preparation of stomach antigen from monkey, guinea-pig, rat, and rabbit.

4. Crude homogenates (20% w/v in buffered saline) were prepared from the pyloric mucosa, duodenum, jejunum, ileum, colon, liver, and kidney obtained at post-mortem examination from a stillborn full-term infant.

Antibody Tests

Antibodies to Intrinsic Factor (I.F.)

Inhibition of I.F. activity in vivo.—The method used was that described previously (Taylor, 1959) in which the effect of pernicious anaemia sera on hog I.F.-mediated absorption of labelled vitamin B_{12} is measured in patients with pernicious anaemia. The test was regarded as positive when the inhibition of vitamin-B_{12} absorption was greater than 15%.

Electrophoretic Retention Test.—Human I.F.-B_{12} complex (50 μl.) was mixed with 20 μl. of serum to be tested and electrophoresed on 4 cm. Whatman No. 3 MM paper strip in barbiturate buffer (pH 8.6, I=0.1) for 16 hours at 2 mA./strip. After drying, the radioactivity was scanned in a strip counter and the globulin bands were identified by staining with azocarmine B. An arbitrary line was drawn 2 cm. behind the trailing edge of the β-globulin zone and the retention of I.F.-B_{12} complex was expressed as the percentage of the total radioactivity remaining behind this line (Fig. 1). A

normal control serum was tested with each electrophoretic run to establish the baseline. With the I.F.-B_{12} preparation used for this study normal sera gave retention values of 25% (S.D. of mean 4%). Abnormal

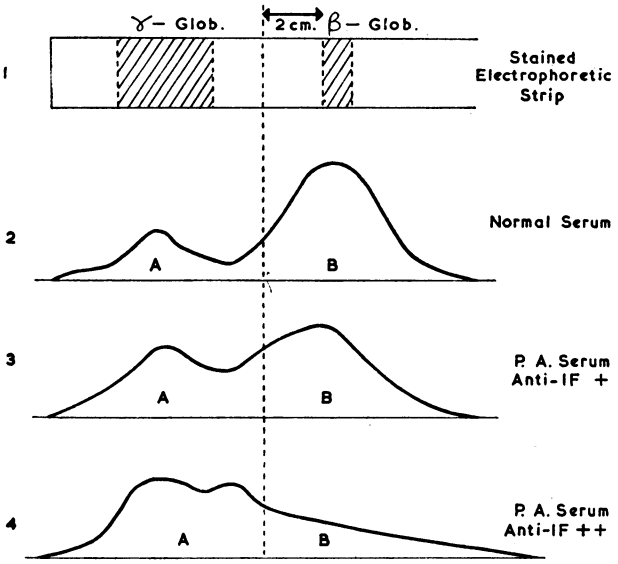

FIG. 1.—(1) Electrophoretic strip of mixture of serum with ^{58}Co-labelled vitamin-B_{12}-intrinsic-factor complex stained to show location of β- and γ-globulin fractions. The position of the arbitrary dividing line 2 cm. behind the β-band is indicated by the vertical dotted line. (2), (3), and (4) Radioactive scans of similar electrophoretic strips obtained with normal, weakly positive, and strongly positive anti-I.F. pernicious anaemia sera respectively. The retention of ^{58}Co activity in the γ-globulin region increases with the amount of anti-I.F. antibody present in the serum.

retention was rated as + for values between 33 and 55% and ++ for values in excess of this. The normal values must be established for each I.F.-B_{12} preparation, since the relative proportions of the two components may differ considerably.

Radioactive Co-precipitation Test.—0.02 ml. of human I.F.-B_{12} preparation was incubated with 0.05 ml. of 1/5 dilution of serum at 37° C. for 30 minutes and kept in the cold for 18 hours. The complex of antibody with I.F.-B_{12} was precipitated by addition of 0.5 ml. of a rabbit anti-human γ-globulin serum. After flocculation the immune precipitate was spun down, washed, and counted in a scintillation well-counter. With normal sera, up to 1.6% of the total radioactivity was co-precipitated. With pernicious anaemia sera containing anti-I.F. antibodies, up to 60% of the radioactivity was co-precipitated.

Ouchterlony Gel Diffusion-precipitation.—Human I.F.-B_{12} complex was set up against undiluted pernicious anaemia and control sera, using 1% agar in buffered saline at *p*H 7.2. Diffusion was continued for three days at room temperature. The gel disks were then dried. Kodak AR50 stripping film was applied for 10 days in cassettes and then developed.

Antibodies to Cytoplasm of Gastric Mucosal Cells

Complement-fixation Test (C.F.T.).—The gastric subcellular preparation described above was used as the antigen in the C.F.T., performed by the semi-micro-method of Donnelley (1951) in "perspex" trays with 2 M.H.D. of complement. Serial dilutions of the sera from 1/4 to 1/512 were tested and the titre was taken to be the highest dilution giving over 50% haemolysis inhibition.

Immunofluorescent Tests.—Fresh gastric mucosa obtained from patients of blood group 0 at operation for gastric or duodenal ulcer or gastric carcinoma was washed free of mucus with normal saline and frozen on a copper plate applied to a block of solid carbon dioxide. It was found helpful to accentuate the mucosal rugae so that the frozen block consisted of a folded loop of mucosa. The gastric glands were sectioned longitudinally. 6μ sections were cut in a cryostat and dried in air without fixation, since alcohol destroyed the cytoplasmic antigen. Test sera were applied for 30 minutes, the slides were rinsed with Coons' buffered saline and soaked in buffer for 10 minutes, following which rabbit anti-human γ-globulin fluorescein conjugate was applied for 20 minutes. After a second 10-minute wash the sections were mounted in 50% glycerol and examined by ultra-violet microscopy (Osram HB 200, Zeiss microscope). Fixed and unfixed thyroid sections were similarly treated for the detection of antibodies to thyroid colloid and cytoplasm as well as antinuclear factors. For convenience the three sections were applied to the same slide.

Results

Intrinsic Factor Antibodies

Thirty sera from patients with pernicious anaemia were examined *in vivo* for their ability to inhibit I.F. activity and *in vitro* for their effect on the electrophoretic mobility of the I.F.-B_{12} complex. The results of the two tests broadly paralleled each other, as may be seen in Fig. 2. Approximately 40% of cases tested had anti-I.F. antibodies. All sera giving a strongly positive result in the electrophoresis test inhibited I.F. activity *in vivo* and virtually all sera negative *in vitro* were also negative by the *in vivo* test. Three sera inhibited I.F. activity

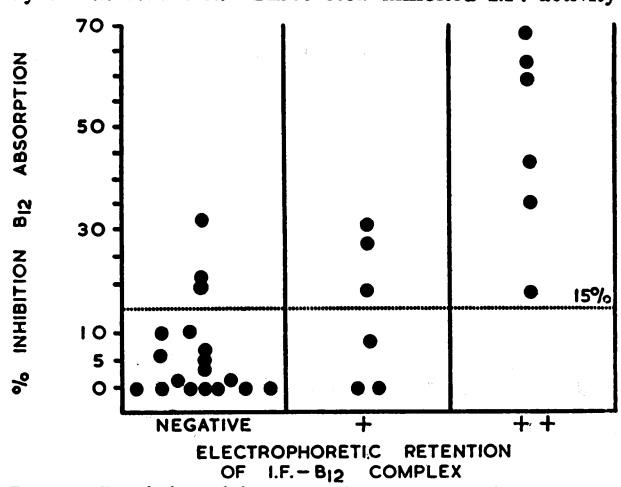

FIG. 2.—Correlation of *in vivo* and *in vitro* tests for auto-antibodies to I.F. Electrophoretic retention is graded on the basis of the degree of reduction in the electrophoretic mobility of I.F.-B_{12} complexes in the presence of pernicious anaemia serum.

and gave negative results in the electrophoretic test In these patients the *in vivo* test had been performed three years previously, so that a reduction in antibody titre may have occurred in that interval. The three sera positive by electrophoresis only were examined at the same time by both methods; this suggests that the electrophoretic test may be somewhat more sensitive under the conditions used, or perhaps that substances other than I.F. are also binding vitamin B_{12}.

The radioactive co-precipitation test was applied to 12 pernicious anaemia sera also examined by electrophoresis. In nine cases the results agreed, being positive

in five and negative in four, while three sera positive by electrophoresis (one strongly so) gave a negative result in the co-precipitation test, suggesting that the electrophoretic method has the greater sensitivity.

Ouchterlony gel diffusion tests gave inconclusive results.

Cytoplasmic Antibodies

Complement fixation.—Of 143 pernicious anaemia sera tested by C.F.T. against the particulate stomach antigen, 62% gave positive results with titres up to 1/256 (Table I). The control group gave 4% positive reactions.

The antigen was localized predominantly in the mitochondrial and microsomal fractions obtained by differential centrifugation of sucrose homogenates of fundal mucosa. Nuclear and supernatant fractions were of low activity. Combined mitochondrial–microsomal fractions gave clear-cut results and were found to be a better antigen than total homogenates.

Immunofluorescence.—Positive fluorescent staining of frozen unfixed sections of human gastric mucosa was obtained with 86% of 143 cases of pernicious anaemia and with 11% of 100 controls (Table I). Staining was localized to the cytoplasm of the *parietal* (acid-producing) cells, which were identified by their distribution (Fig. 3 A and B) and by comparison with serial sections stained with haematoxylin and eosin. The specific fluorescence covered the entire cytoplasm and was granular in appearance, particularly with strongly positive sera. Absorption of sera with appropriate red blood cells and the use of blood group O stomach as substrate precluded the possibility that staining was due to blood-group antibodies. Staining was abolished by pretreatment of the sections with ethanol or methanol.

It is worth noting that pernicious anaemia sera with high anti-I.F. activity did not appear to stain any other cell type in the fundal mucosa. This suggests either that localization of I.F. in the tissue is not possible by this means or that the parietal cell is also the site of I.F. production. A separation of anti-I.F. and gastric cytoplasmic antibodies may help to resolve this problem.

Relation of C.F.T. to Immunofluorescence

There was close correlation between complement fixation and immunofluorescent staining (Fig. 4). All sera giving positive C.F.T. stained the parietal cell cytoplasm and all sera negative by immunofluorescence were negative by C.F.T. Clearly the fluorescent antibody test is the more sensitive of the two.

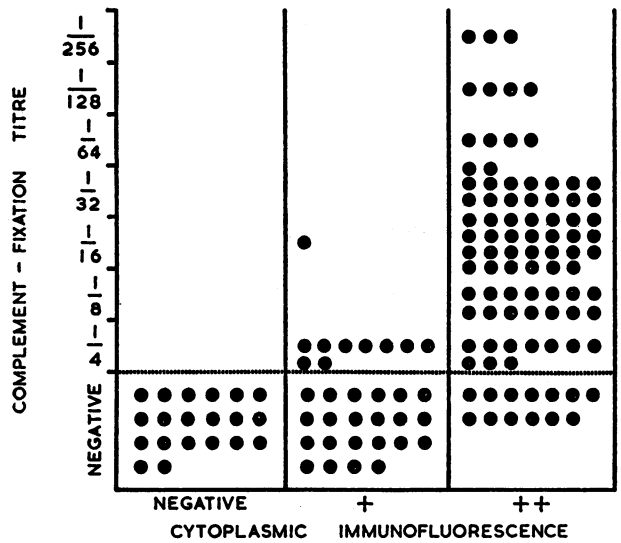

FIG. 4.—Correlation of the results of complement-fixation and immunofluorescent tests for detection of parietal cell antibodies in pernicious anaemia patients.

Antigen Specificity

Organ specificity.—Pernicious anaemia sera with high-titre antibodies to parietal cells gave negative results when tested by C.F.T. against extracts of human pyloric, duodenal, ileal, and colonic mucosa. The organ specificity of the antibodies was further established by testing strongly positive sera against extracts of stomach, liver, and kidney taken from a single individual. All sera

TABLE I.—*Incidence and Titres of Gastric Parietal Cell Cytoplasmic Antibodies in Pernicious Anaemia Patients and Controls*

	No. Tested	Total Positive	No. of Patients with Gastric Cytoplasmic Antibodies							
			C.F.T. < Immunofluor. Positive	C.F.T. Titres						
				1/4	1/8	1/16	1/32	1/64	1/128	1/256
Pernicious anaemia	143	123 (86%)	35 (24%)	19 (13%)	14 (10%)	28 (20%)	16 (11%)	4 (3%)	4 (3%)	3 (2%)
Controls	100	11	7	2	0	0	1	0	0	1

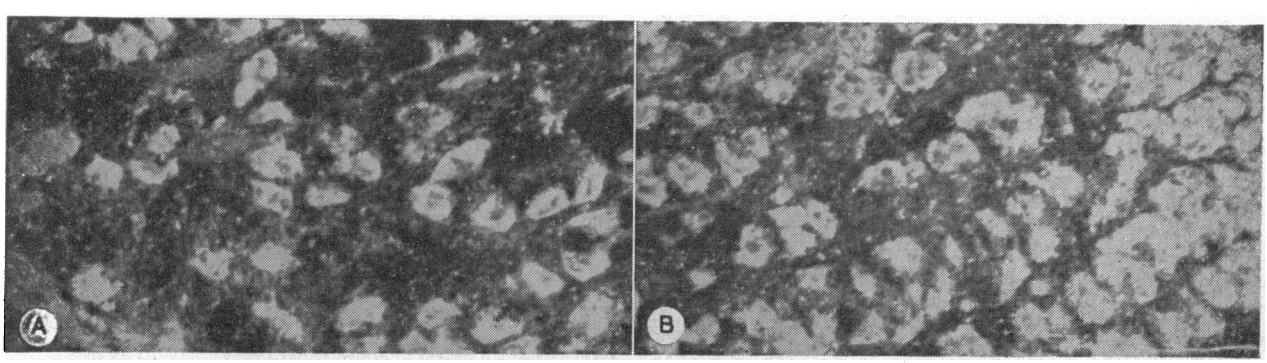

FIG. 3.—Immunofluorescent staining of gastric parietal cells by pernicious anaemia serum. Fresh-frozen sections of human group O gastric mucosa treated with serum of a pernicious anaemia patient, followed by anti-human γ-globulin fluorescin conjugate; viewed by ultra-violet light. The specific staining is confined to the parietal cell cytoplasm. A, High-power view of longitudinal section of gastric glands with underlying muscularis mucosae showing isolated parietal cells with cytoplasmic staining at the base of the glands. Pepsin-secreting (chief) cells are unstained. B, Same section; contiguous field showing maximal density of parietal cells. The nuclei are unstained.

were tested by C.F.T. against toxic thyroid tissue and rat-liver homogenates ; although some sera gave positive results owing to the concomitant presence of either thyroid cytoplasmic or non-organ specific (A.I.C.F.) antibodies, these reactions were independent of those obtained with gastric mucosa. The separate identities of the thyroid and gastric cytoplasmic antibodies were evident from their independent behaviour in the C.F.T. and immunofluorescent test obtained with the two organs. The lack of correlation is shown in Fig. 5.

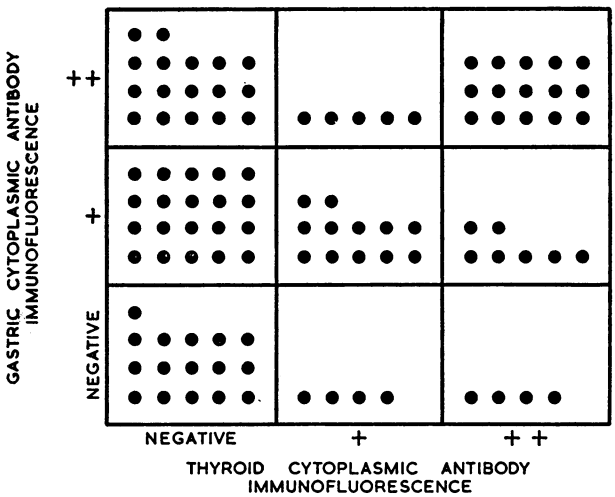

FIG. 5.—Independence of gastric and thyroid cytoplasmic antibodies in pernicious anaemia sera, as shown by the lack of correlation.

Species specificity.—Crude homogenates of gastric mucosa of monkey, rabbit, guinea-pig, and rat all cross-reacted in the C.F.T. with a pernicious anaemia serum giving a titre of 1/256 with human stomach and negative results with human and rat liver. The titres with monkey, rat, and rabbit stomach were 1/8 and with guinea-pig stomach 1/16. The same serum antibody titres were obtained over a wide range of antigen concentrations.

Characteristics of the Antibody

A high-titre pernicious anaemia serum was fractionated on a column of D.E.A.E.-cellulose. The complement-fixing antibodies were entirely recovered in the 7*S* γ-globulin breakthrough peak obtained in 0.02M phospate buffer, *p*H 6.3. The C.F. antibodies were relatively stable to heating at 63° C. for 10 minutes. The C.F.T. titres of 6 pernicious anaemia sera were unaffected by treatment with 0.1 M 2-mercaptoethanol.

Relation of Parietal Cell and I.F. Antibodies

Pernicious anaemia sera containing high-titre cytoplasmic antibodies did not fix complement with human gastric juice containing I.F. The independence of the two immune systems is further emphasized by the fact that some sera having no anti-I.F. activity had cytoplasmic antibodies, often in high titre (Fig. 6). None the less, the sensitive immunofluorescent test was positive in all but one of the patents showing anti-I.F. activity, and, further, of 17 pernicious anaemia sera without cytoplasmic antibodies only one proved to have anti-I.F. antibodies when tested by electrophoresis. This suggests that the appearance of cytoplasmic antibodies may precede that of anti-I.F. antibodies, but only serial studies in the same patients will resolve this problem.

Incidence of Antibodies and Duration of Disease

The data on I.F. and gastric C.F. antibodies have been examined in relation to the period between establishing the diagnosis of pernicious anaemia and obtaining the

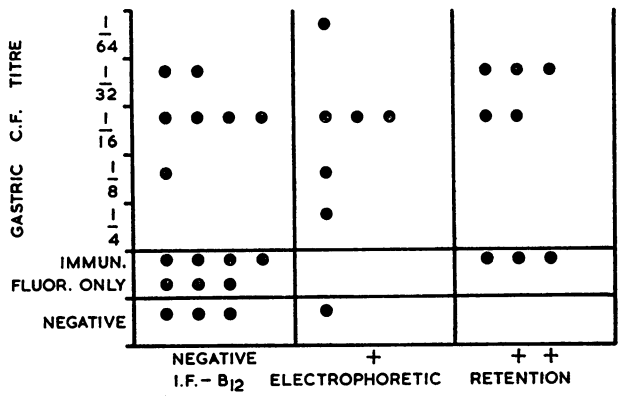

FIG. 6.—Correlation of results of tests for parietal cell and I.F. antibodies in pernicious anaemia patients, showing their independence.

test sample of serum for each patient, since the duration of evolution of the gastric lesion in pernicious anaemia, which would be a more appropriate index, is unknown. The results are shown in Table II, and it can be seen that the presence or absence of either antibody is independent of the duration of overt disease.

TABLE II.—*Incidence of Antibodies and Duration of Disease*

Test	No. of Patients	Duration of Diagnosed P.A. (Years)		
		Mean	Range	S.D.
Anti-I.F. *in vivo* +	12	4·1	0–19	5·75
Anti-I.F. *in vivo* −	18	3·3	0–18	4·9
Anti-I.F. *in vitro* +	14	6·3	0–19	5·4
Anti-I.F. *in vitro* −	18	5·2	0–18	4·8
Gastric C.F.T. +	19	5·8	0–18	5·4
Gastric C.F.T. −	13	5·2	0–19	4·8

Discussion

The results presented show clearly that in the sera of patients with pernicious anaemia at least two distinct antibodies may be found. The first, present in about 40% of cases, inhibits biological activity of hog I.F. and may be detected *in vitro* by an electrophoretic retention test using a complex of human I.F. and labelled cyanocobalamin. The second antibody reacts against a particulate component of the cytoplasm of the gastric parietal cell and may be detected by complement fixation or by immunofluorescence.

The results of biological testing for anti-I.F. activity confirm earlier observations by Taylor (1959b) and Schwartz (1960). In addition Schwartz observed that pernicious anaemia sera which inhibited hog I.F. also inhibited human and rat I.F., and Jeffries *et al.* (1962) have likewise shown inhibition of human I.F. by pernicious anaemia sera. Taylor and Morton (1958) showed cross-reactions between hog and human I.F. with rabbit antisera prepared against either, underlining the antigenic relationships between the I.F. of the different species tested.

The electrophoretic retention test was first used for the detection of heterologous I.F. antibodies by Lowenstein *et al.* (1961) and was then applied to the study of the human auto-antibody by Jeffries *et al.* (1962). It has proved to be a rather more sensitive method than the

in vivo test, and since it involves the use of far less material, time, and labour it is clearly preferable. We have found that electrophoresis on paper, followed by automatic strip scanning, is more convenient than the starch gel method for the examination of large numbers of sera. However, using this test, a preliminary examination of a serum with high anti-hog I.F. activity by the *in vivo* test and with anti-human I.F. activity by electrophoresis gave a negative result with a hog I.F.-B_{12} complex. This suggests that some species specificity may obtain in the *in vitro* test which is not apparent in the biological test. This problem is being examined.

C.F. antibodies to a saline extract of human gastric fundal mucosa have already been described (Irvine *et al.*, 1962) and our own results confirm those of the Edinburgh group, who found a positive C.F.T. in 75% of 41 cases. Using a mitochondrial–microsomal fraction of fundal mucosa, which gave more clear-cut results than the crude homogenate, we found the sera of 88 out of 143 patients (62%) to be positive at 1/4 or higher titre. The immunofluorescent test gave a much higher percentage of positives (86% of 143 patients). The complete correlation obtained shows that the two methods are measuring the same antibody. Apart from its greater sensitivity, the immunofluorescent test also has the advantage that non-organ-specific (A.I.C.F.) antibodies do not interfere, whereas the organ specificity of a positive C.F.T. with stomach has to be verified by testing with liver.

Fluorescent staining of the fundal mucosa was localized to the cytoplasm of the parietal cells. The lack of correlation between the incidence of parietal-cell staining and the incidence of thyroid cytoplasmic antibody, and the absence of reactions with other tissues or even other cells in the gastric mucosa, show that the parietal-cell antibody is organ-specific and, indeed, cell-specific.

If one may assume that the presence of auto-antibodies reflects an underlying immunological pathogenesis, the depletion of parietal cells and diminished acid production seen in pernicious anaemia would be expected; in the same way, hypothyroidism is the end-result of non-goitrous autoimmune thyroiditis where antibody to the acinar epithelial cells is involved.

It was previously suggested that autoimmune diseases fall broadly into two main groupings—one associated with organ-specific antibodies and exemplified by lymphoid thyroiditis (including Hashimoto's disease), and the other characterized by the presence of a variety of non-organ-specific antibodies, as seen in systemic lupus erythematosus (Hijmans *et al.*, 1961). The high incidence of antibodies specific for stomach in pernicious anaemia and the localization of the lesion to this organ places this disease clearly in the organ-specific group. There are many other points of similarity between pernicious anaemia and autoimmune thyroiditis, particularly its atrophic variant, primary myxoedema, which we have summarized in Table III. Both are chronic diseases in which hereditary factors almost certainly operate, and in which sex incidence and age of onset are similar. The histological appearances of gastric mucosa in atrophic gastritis and thyroid in myxoedema have much in common, the round-cellular infiltration being most striking in both diseases. In addition it may be seen from Table III that there are many more similarities than differences between the immune systems concerned in the two conditions.

Recently experimental work has provided a further link between them. The production of thyroiditis by autoimmunization in animals is well established (Rose and Witebsky, 1956; Jones and Roitt, 1961), and, using a similar technique, Hennes *et al.* (1962) have reported the production of gastritis in a limited number of dogs.

TABLE III.—*Comparison of Some Features of Pernicious Anaemia and Myxoedema (Autoimmune Thyroiditis)*

	Myxoedema	Pernicious Anaemia
Clinical	Frequent clinical association Both chronic diseases Familial Similar age of onset Commoner in females	
Pathological	Tissue destruction and atrophy Infiltration with lymphocytes, plasma cells, and histiocytes Loss of normal cellular function	
Immunological: Auto-antigens:	Normal body constituents	
Particulate	Complement fixing Demonstrable by cytoplasmic immunofluorescence Destroyed by ethanol Organ specific	
	Narrow species specificity	Wide species specificity
Soluble*	Non-complement fixing Both cellular secretory products	
Auto-antibodies	7S γ-globulins Low incidence of non-organ-specific antibodies Frequent coexistence of organ-specific antibodies	

* Thyroglobulin and intrinsic factor respectively.

The clinical and immunological similarities between pernicious anaemia and thyroiditis strongly suggest that the mechanisms underlying the pathogenesis of these two diseases are identical and are probably of an immunological nature.

The high percentage of positive results with pernicious anaemia sera, using immunofluorescence, suggests the possibility that such a test may be of value in the diagnosis of pernicious anaemia. In sera from pernicious anaemia patients aged 60 years or less 93% were positive, whereas the incidence in matched controls was only 5% (Table IV). To establish the diagnostic significance of this test, groups of patients with other diseases, particularly those associated with vitamin-B_{12} deficiency due to small-bowel malabsorption, will have to be studied. Above the age of 60 there is an apparent increase in the incidence of gastric antibodies in the controls (16%). The difference between the two age-groups does not reach an accepted level of statistical significance ($\chi^2=2.9$ uncorrected, $n=1$; $P>0.05$). If this trend were confirmed by studies in a larger group it might reflect an increasing incidence of gastritis with age, which has been suggested by many (Williams, 1950) though denied by others (Palmer, 1954).

TABLE IV.—*Relation of Age to Incidence of Gastric Antibodies in Pernicious Anaemia*

Age in years:	⩽60	>60
Pernicious anaemia	39/42 (93%)	44/58 (76%)
Matched controls	2/42 (5%)	9/58 (16%)

Summary

The sera of 143 patients with pernicious anaemia have been tested for auto-antibodies to intrinsic factor and to gastric mucosa.

Anti-I.F. antibodies were estimated by *in vivo* inhibition of vitamin B_{12} absorption, by electrophoretic retention of I.F.-B_{12} complex, and by radioactive co-

precipitation. These three methods correlate well. The electrophoretic method was found to be the most suitable for the study of this antibody.

Antibodies to gastric mucosa were demonstrated by complement fixation and immunofluorescence. The two methods gave good correlation, the latter being more sensitive.

The antigen was localized in the cytoplasm of the parietal cells and was recovered in the particulate fraction of gastric mucosal homogenates.

The complement-fixing antibody is a 7*S* γ-globulin, which is specific for parietal cells and cross-reacts with gastric mucosal extracts of other species.

The parietal-cell antibody is distinct from anti-I.F.

Of patients with pernicious anaemia, 44% had auto-antibodies to I.F. The incidence of parietal-cell antibodies was 86%. This compares with 11% in controls matched for age and sex.

The immunofluorescent test may have important diagnostic applications, particularly for pernicious anaemia patients under the age of 60, where the incidence of positive reactions was 93%.

Close parallels between pernicious anaemia and auto-immune thyroiditis are drawn on the basis of clinical and immunological features.

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REFERENCES

Abels, J., Jansz, A., Woldring, M. G., Arends, A., and Nieweg, H. O. (1962). Vitamin B_{12} and Intrinsic Factor. 2nd Europ. Symposion, 1961, p. 623. Enke, Stuttgart.
Bastenie, P. (1937). *Arch. int. Méd. exp.*, **12**, 1.
Doniach, D., and Roitt, I. M. (1957). *J. clin. Endocr.*, **17**, 1293.
Donnelley, M. (1951). *Aust. J. exp. Biol. med. Sci.*, **29**, 137.
Hennes, A. R., Sevelius, H., Lewellyn, T., Joel, W., Woods, A. H., and Wolf, S. (1962). *Arch. Path.*, **73**, 281.
Hijmans, W., Doniach, D., Roitt, I. M., and Holborow, E. J. (1961). *Brit. med. J.*, **2**, 909.
Irvine, W. J., Davies, S. H., Delamore, I. W., and Williams, A. W. (1962). Ibid., **2**, 454.
Jeffries, G. H., Hoskins, D. W., and Sleisenger, M. H. (1962). *J. clin. Invest.*, **41**, 1106.
Jones, H. E. H., and Roitt, I. M. (1961). *Brit. J. exp. Path.*, **42**, 546.
Lowenstein, L., Cooper, B. A., Brunton, L., and Gartha, S. (1961). *J. clin. Invest.*, **40**, 1656.
Palmer, E. D. (1954). *Medicine (Baltimore)*, **33**, 199.
Rose, N. R., and Witebsky, E. (1956). *J. Immunol.*, **76**, 417.
Schwartz, M. (1960). *Lancet*, **2**, 1263.
Taylor, K. B. (1959a). *Haemat. lat. (Milano)*, **2**, 181.
—— (1959b). *Lancet*, **2**, 106.
—— and Morton, J. A. (1958). Ibid., **1**, 29.
Tudhope, G. R., and Wilson, G. M. (1960). *Quart. J. Med.*, **29**, 513.
—— —— (1962). *Lancet*, **1**, 703.
Williams, A. W. (1950). *Brit. med. J.*, **1**, 102.
Williams, E. D., and Doniach, I. (1962). *J. Path. Bact.*, **83**, 255.

Over 60,000 students from 140 countries were studying in Britain in 1961 to 1962 according to *Overseas Students in Britain*, a new edition of which has been issued by the London Conference on Overseas Students. Just under a quarter of the total—13,293—were in universities, and of these 2,138 were reading medicine. (British Council, London Overseas Students Department, 3 Hanover Street, London W.1, price 1s. 6d.)

THYROID AUTO-ANTIBODIES IN PERNICIOUS ANAEMIA

BY

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The reported impairment of vitamin B_{12} absorption in three out of seven patients with hypothyroidism prompted Tudhope and Wilson (1960), working in Sheffield, to investigate the incidence of pernicious anaemia in 166 patients with hypothyroidism. They found that it was surprisingly high—7.8% in the whole series and 10.3% if those with previous thyroidectomy or with thyrotoxicosis treated with radioiodine or *x*-irradiation were excluded. The present investigation approaches this interesting association from a different aspect—namely, the incidence of thyroid disease in pernicious anaemia.

The modern view of hypothyroidism is that "classical Hashimoto's thyroiditis and 'primary' hypothyroidism are clinical variants of the same pathological process" (Buchanan *et al.*, 1961). Thyroid biopsy is not justifiable in an investigation of this kind, and not all cases of Hashimoto-type thyroiditis suffer from hypothyroidism. Thyroid auto-antibodies in high titre are seldom found except in the serum of persons with this type of thyroiditis (Fulthorpe *et al.*, 1961). It was considered, therefore, that examination of the serum of patients with pernicious anaemia for these antibodies was the most appropriate line of inquiry to pursue.

Patients Studied

Serum was obtained from 78 patients with pernicious anaemia and from 78 controls. The former had all presented originally to one of us as untreated cases and the diagnosis was made on the basis of (1) megaloblastic bone-marrow, (2) histamine-fast achlorhydria, and (3) satisfactory response of the anaemia to vitamin B_{12} therapy. In many cases the serum vitamin B_{12} level was estimated and the Schilling test carried out, providing further confirmatory evidence for the diagnosis. All but six of the cases of pernicious anaemia were on maintenance therapy and attended a follow-up clinic. One, a male, was also being treated for myxoedema of long duration, and another, a female, was suffering from rheumatoid arthritis. Age and sex of the patients are shown in Table I. The ratio of females to males was approximately 2 to 1. Only four patients were under 40 years of age.

The control sera were obtained from patients with a variety of diseases, listed in Table II, who did not suffer from thyroid disease or have an enlarged thyroid gland, and who were not selected in any other way except as necessary to ensure matching with the pernicious-

TABLE I.—*Age and Sex of Pernicious Anaemia Patients Studied*

	20–29	30–39	40–49	50–59	60–69	70–79	80+	Total
Males	2	—	4	4	9	6	—	25
Females	—	2	6	11	15	17	2	53
Total	2	2	10	15	24	23	2	78