was obvious that very substantial relief of pain was obtained in most cases. Each patient was also carefully questioned, but, in order that the results could be judged with the least possible bias, no comment was made to the patient at the time of the injection, save to explain that an examination was to be made. Ten minutes after the injection the result was assessed by asking the patient, "How are the pains?" without giving any suggestion of expected relief. When recorded and analysed the answers indicated "complete" or "very good" relief of pain in 71%, "partial relief" in 25%, and "no relief" in 4%.

These results may be compared with those of other workers, which for this purpose are expressed under similar headings, and on a percentage basis to the nearest unit.

| | Freeman *et al.* | Davis *et al.* | Aldridge *et al.* | Present Series |
|---|---|---|---|---|
| Complete or very good relief | 86 | 90 | 76 | 71 |
| Partial relief .. .. | 4 | — | 19 | 25 |
| No relief .. .. .. | 10 | — | 5 | 4 |

*Contraindications.*—The possibility of sensitivity of the patient to the local anaesthetic agent used must be kept in mind. We have not encountered this trouble in our series, but it is obvious that the initial injection should be made slowly and with the patient kept under careful supervision.

### Side-effects

In one case we noted a temporary foetal bradycardia after the injection; in another case, an early one of the series, the following undesirable effect was observed—presumably the result of an inadvertent intravenous injection of the anaesthetic agent, which is a hazard common to all methods of local anaesthesia.

The patient was well established in labour but was experiencing a great deal of pain. A paracervical injection was satisfactorily given on the left side. When, however, the needle was transferred to the right side some blood was drawn into the barrel of the syringe. The needle was then reinserted, but after the injection of a few millilitres of solution the patient became for a few minutes pale, dyspnoeic, and distressed. Incidentally, there was another interesting feature in this case. Only the left side had been satisfactorily injected; the patient later declared, quite spontaneously, that labour pains had completely disappeared from that side, but not from the other side of the body.

Apart from the complication referred to above we have had no significant trouble attributable to the procedure. In other recent reports the following untoward effects have been recorded, which, however, seem to be attributable to errors in method or technique.

Freeman *et al.* (1956) recorded foetal bradycardia in 2 or 3% of their series. They also recorded one foetal death due to maternal collapse; this was consequent upon the injection of 45 ml. of 1% "cyclaine" local anaesthetic—a dose which seems needlessly large, especially if the adrenaline was omitted and rapid absorption thus favoured. Injection of a nerve root was recorded by the same author. In this case pain and weakness occurred for three months before complete recovery took place. The drug used was efocaine, which is now recognized to carry a danger of causing prolonged neuritis, and which for that reason is seldom now employed.


### Summary

A simple method of nerve block is described by which pain in the first stage of labour may be relieved for a period of one to two hours.

The results in a series of 100 administrations are described. Complete or almost complete relief of pain was obtained in 71% of cases, and partial relief in a further 25%.

Paracervical block is of considerable use towards the end of the first stage of labour, not only because it relieves the patient of her suffering but also because it enables the obstetrician to make an adequate examination with much greater ease and precision.


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# AUTOIMMUNE PHENOMENA IN PERNICIOUS ANAEMIA

## SEROLOGICAL OVERLAP WITH THYROIDITIS, THYROTOXICOSIS, AND SYSTEMIC LUPUS ERYTHEMATOSUS


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Attention has recently been drawn to the significant association of thyroid diseases with pernicious anaemia. Tudhope and Wilson (1960) found that nearly 10% of patients with myxoedema had pernicious anaemia, and in a later study (Tudhope and Wilson, 1962) an even higher proportion were shown to have impaired absorption of cyanocobalamin due to a deficiency of intrinsic factor. McNicol (1961) found that 5% of patients with pernicious anaemia were or became thyrotoxic, the majority developing overt thyroid disease at varying periods after diagnosis and treatment of the anaemia. Further support for such a relationship is provided by the finding of an increased incidence of thyroiditis in patients with pernicious anaemia examined at necropsy (Bastenie, 1937; Williams and Doniach, 1962).

There is little doubt that thyroid autoimmunity is strongly implicated in myxoedema and to a less extent in thyrotoxicosis, while recent work has demonstrated autoimmune phenomena in pernicious anaemia (Taylor, 1959; Schwartz, 1960; Jeffries *et al.*, 1962; Irvine *et al.*, 1962; Taylor *et al.*, 1962). It is possible that the


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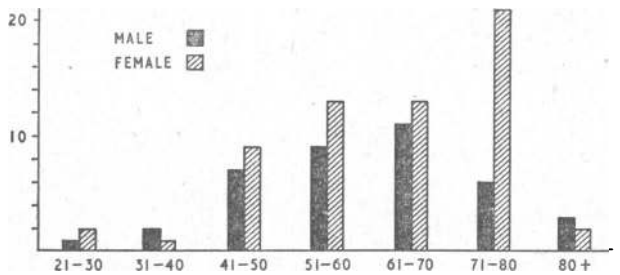
association between disease of the gastric mucosa and of the thyroid gland reflects the closeness of developmental origin of these tissues and that autoimmune mechanisms underlie the pathogenesis of the lesions in both organs. We have therefore determined the incidence of auto-antibodies to various components of the thyroid gland in the sera of patients with pernicious anaemia and related this to the occurrence of clinical thyroid disease. The serological overlap of thyroid and gastric antibodies was further investigated in patients with Hashimoto's disease and thyrotoxicosis. The relationship of these disorders to systemic lupus erythematosus (S.L.E.) was of special interest in view of the apparent defects of immunological homoeostasis in this disease, and we have looked for antinuclear factors (A.N.F.) and non-organ-specific complement-fixing (A.I.C.F.) antibodies in pernicious anaemia and for organ-specific antibodies in S.L.E.

## Materials and Methods

Sera were obtained from 100 pernicious anaemia (P.A.) patients attending the anaemia clinic of the Nuffield Department of Clinical Medicine, Oxford, and 53 further sera were collected at the Middlesex Hospital, thus making a total of 153 pernicious anaemia sera tested for thyroid antibodies, A.N.F. and A.I.C.F. The Oxford patients were unselected, of either sex, and of ages ranging from 27 to 91 years (see Chart). All had been diagnosed by typical changes in the peripheral blood and sternal marrow, by their abnormally low




Age and sex distribution of 100 cases of pernicious anaemia.

absorption of radioactive cobalt-labelled cyanocobalamin, by barium-meal examination, and, in the majority of patients, by assay of vitamin $B_{12}$ activity in the serum. In a number of cases gastric biopsy had also been performed, and tests of intestinal absorption to exclude disease of the small intestine. All were receiving adequate treatment with cyanocobalamin by injection. Each patient was interviewed and questioned specifically about personal and familial thyroid disease, and examination for enlargement of the thyroid gland and for other clinical signs of thyroid disease was made. The Middlesex Hospital pernicious anaemia series included some patients referred for thyroid antibody tests because of overt or suspected thyroid disease; they are therefore partly selected, and for this reason they have not been considered in those clinical and immunological correlations where selection renders them inappropriate.

The sera of 58 thyrotoxic patients sent to the laboratory for thyroid antibody tests were examined for gastric antibodies. Only definite cases with a classical history, clinical examination, and raised $^{131}I$ uptake were included. The S.L.E. sera had been sent for A.N.F. tests, and 61 cases with positive L.E. cell tests were available for gastric antibody tests. Of these, three patients also had Hashimoto goitres and two had an associated thyrotoxicosis with a substantial degree of thyroiditis. One hundred and thirteen Hashimoto cases were tested for gastric antibodies, but the incidence of thyroid antibodies was calculated from a larger series examined previously.

Control sera were obtained from subjects in apparent good health. They were matched for age and sex with the unselected pernicious anaemia series, since the incidence of auto-antibodies shows strong correlations with both factors (Hill, 1961; Rothfield *et al.*, 1962).

The sera were stored at −20° C. for up to two years.

### Serological Tests

1. *Gastric Antibodies.*—Antibodies to the particulate auto-antigen of the parietal cells were detected by complement fixation test (C.F.T.) and by immunofluorescent tests as described in the first part of this study (Taylor *et al.*, 1962).

2. *Thyroid Antibodies.*—The tanned red-cell test (T.R.C.) was used for the detection of thyroglobulin antibodies (Fulthorpe *et al.*, 1961). Antibodies to the "microsomal" antigen of the thyroid cell cytoplasm were detected by C.F.T. as previously described (Roitt and Doniach, 1958) and by immunofluorescence, using unfixed thyrotoxic thyroid sections (Holborow *et al.*, 1959). Antibodies to the second antigen of the acinar colloid were tested for by immunofluorescence, using thyroid sections fixed for three minutes in absolute methanol at 56° C. to retain the colloid.

3. *Non-organ-specific Antibodies.*—A.N.F. were detected by the fluorescent antibody test using a fluorescein-conjugated rabbit anti-human γ-globulin serum applied to alcohol-fixed and unfixed thyroid sections, and unfixed rat-liver sections treated with the serum under test at room temperature. When nuclei stood out as green dots in both thyroid and liver sections, the reaction was graded as weakly positive (wk+), positive (+), and strongly positive (++) according to the intensity of the fluorescence. The titre of wk+ sera was <1/10.

A.I.C.F. antibodies were detected by C.F.T., using as antigen the supernatant from saline homogenates of fresh rat liver after removal of whole cells and nuclei by spinning at 2,000 r.p.m. (800 *g*) for 10 minutes.

### Thyroid-function Tests

These included the following measurements: thyroid uptake 4 and 24 hours after an oral dose of 20 μc. of $^{131}I$; urinary excretion for the period of 6 to 24 hours after the dose; red-cell uptake of [$^{131}I$]tri-iodothyronine *in vitro* (Hamolsky *et al.*, 1959); and plasma protein-bound radioactivity at 48 hours (Hughes and Miller, 1956). In some patients the thyroid uptake test was repeated 24 hours after intramuscular injection of 10 units of thyroid-stimulating hormone (T.S.H.; "thyropar," Armour).

## Results

### Incidence of Clinical Thyroid Disease in Pernicious Anaemia

The clinical findings are summarized in Table I. Of the 100 patients in the Oxford series, seven were thyrotoxic or had received treatment for toxic thyroid disease within the previous 10 years, two had myxoedema, and six had either diffuse or nodular swelling of the thyroid gland without clinical evidence of thyroid dysfunction. Eleven patients in whom there was no clinical thyroid disease had one or more near

relatives (sibling, parent, offspring, cousin) with thyroid disease.

TABLE I.—*Incidence of Overt Thyroid Disease in Patients with Pernicious Anaemia*

| | No. of Cases | Thyrotoxicosis | Primary Myxoedema or Hashimoto's Disease | Non-toxic Goitre | Normal Thyroid: Relative with Thyroid Disease |
|---|---|---|---|---|---|
| Oxford .. | 100 | 7 | 2 | 6 | 11 |
| Middlesex | 53 | 6 | 8 | 5 | Not known |

Of the Middlesex series of 53 cases, 19 had thyroid disease.

**Thyroid Antibodies in Pernicious Anaemia**

The incidence of antibodies to the three known thyroid auto-antigens was determined in the sera of 100 unselected pernicious anaemia patients and 100 matched controls. Table II presents the results for the microsomal antibody detected by C.F.T. and the more sensitive and specific immunofluorescent test. This antibody was present in 47% of the P.A. patients and in 13% of the controls, the difference being significant ($\chi^2=29.724$ ; n=3 ; P<0.001). The trend in both groups is towards a greater incidence and higher titres in females. The figures for 576 patients with Hashimoto's disease are included for comparison. These show that the antibody is found in virtually all cases, and is present in high concentration in over 70%.

The results for thyroglobulin antibodies in the same three groups are shown in Table III. The tanned red-cell test was positive in 29% of pernicious anaemia patients and 8% of matched controls, the difference being significant ($\chi^2=15.366$ ; n=2 ; P<0.001). Both sexes show a similar incidence. The immunofluorescent test for antibodies to the *non-thyroglobulin* colloid antigen was positive in a high proportion of both pernicious anaemia and control sera owing to the preponderance of elderly subjects, but there was a significantly higher percentage of strongly positive reactions among the female pernicious anaemia patients (Table (IV).

Since we have shown that an appreciable proportion of our unselected pernicious anaemia patients had overt thyroid disease, we have separated them and the Middlesex Hospital cases into two groups to compare the incidence of thyroid antibodies in the presence and absence of thyroid disease. Of 123 pernicious anaemia patients with normal thyroid function, 60% gave positive results at least in one test and 37% had antibodies to both colloid and cytoplasm (Table V). This compares with value of 25% and 8% respectively for the control group. The presence of thyroid disease in pernicious anaemia was associated with an even higher proportion of positive results, since 57% of the patients had more than one thyroid antibody. The striking difference between uncomplicated pernicious anaemia and controls with respect to the presence of multiple thyroid antibodies is of particular interest, since this

TABLE IV.—*Incidence of Antibodies to Second Antigen of Thyroid Colloid in Pernicious Anaemia, Healthy Matched Controls, and Hashimoto's Disease*

| | No. Tested | | Colloid Immunofluorescence Test | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total Positive | | + | | ++ | |
| | M | F | M | F | M | F | M | F |
| Pernicious anaemia | 38 | 55 | 10 (26%) | 25 (45%) | 9 (23%) | 13 (23%) | 1 (3%) | 12 (22%) |
| Healthy controls | 39 | 60 | 6 (15%) | 20 (33%) | 6 (15%) | 18 (30%) | 0 (0%) | 2 (3%) |
| Hashimoto's disease | 8 | 203 | 8 (99%) | 201 | Not subdivided—most patients strongly positive | | | |

Statistical significance of differences between pernicious anaemia and healthy control sera giving strong immunofluorescence (++) and combining the sexes : $\chi^2=9.52$ ; degrees of freedom=1; P<0.01. Significant.

Antibodies to second antigen of the colloid could only be assessed in patients having T.R.C. titres below 1/2,500, since higher tires of thyroglobulin antibodies produce fluorescent staining of the colloid unless the sera are first absorbed with thyroglobulin.

TABLE V.—*Incidence of Thyroid Antibodies in Pernicious Anaemia Patients With and Without Overt Thyroid Disease*

| | No. Tested | Thyroid Antibodies | | | |
|---|---|---|---|---|---|
| | | Cytoplasmic* | Colloid† | Cytoplasmic and Colloid | Total Patients Positive |
| P.A. without overt thyroid disease .. | 123 | 56 (45%) | 50 (41%) | 46 (37%) | 74 (60%) |
| P.A. with thyroid diseases .. | 30 | 20 (67%) | 22 (73%) | 17 (57%) | 26 (87%) |
| Healthy controls | 100 | 13% | 21% | 8% | 25% |

* Immunofluorescence.
† Antibodies to colloid include T.R.C. ≥1/25 and immunofluorescence of colloid.

TABLE II.—*Incidence and Titres of Thyroid Microsomal Antibodies in Pernicious Anaemia, Matched Healthy Controls, and Hashimoto's Disease*

| | No. Tested | | Total Positive | | | | C.F.T.<1/4 Immunofluorescence Positive | | | | C.F.T. Titres | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | 1/4–1/16 | | | | 1/32–1/512 | | | |
| | M | F | M | | F | | M | | F | | M | | F | | M | | F | |
| | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Pernicious anaemia .. | 39 | 61 | 13 | 33 | 34 | 56 | 6 | 15 | 10 | 16 | 5 | 13 | 12 | 20 | 2 | 5 | 12 | 20 |
| Healthy controls .. | 39 | 61 | 5 | 13 | 8 | 13 | 5 | 13 | 3 | 5 | 0 | | 3 | 5 | 0 | | 2 | 3 |
| Hashimoto's disease .. | 46 | 530 | 46 | 100 | 528 | 99·6 | 5 | 11 | 80 | 15 | 3 | 7 | 74 | 14 | 38 | 82 | 374 | 70·6 |

Statistical significance of the differences between pernicious anaemia and healthy controls, combining the sexes: $\chi^2=29.724$; degrees of freedom=3; <0·001. Significant.

TABLE III.—*Incidence and Titres of Thyroglobulin Antibodies in Pernicious Anaemia, Matched Healthy Controls, and Hashimoto's Disease*

| | No. Tested | | Tanned-cell Agglutination Titres | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total Positive | | | | 1/5–1/250 | | | | 1/2,500–1/25,000 | | | | <1/250,000 | | | |
| | M | F | M | | F | | M | | F | | M | | F | | M | | F | |
| | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Pernicious anaemia .. | 39 | 61 | 12 | 31 | 17 | 28 | 10 | 26 | 10 | 16 | 2 | 5 | 4 | 7 | 0 | | 3 | 5 |
| Healthy controls .. | 39 | 61 | 3 | 8 | 5 | 8 | 3 | 8 | 4 | 6·5 | 0 | | 1 | 1·5 | 0 | | 0 | |
| Hashimoto's disease .. | 46 | 530 | 41 | 89 | 415 | 78 | 3 | 6·5 | 88 | 16 | 7 | 15 | 93 | 17 | 31 | 67·5 | 234 | 45 |

Statistical significance of the differences between pernicious anaemia and healthy controls, combining the sexes, and also the reactions greater than 1/250: $\chi^2=15.366$; degrees of freedom=2; P<0·001. Significant.

combination is a better index of focal lymphadenoid change in the thyroid gland than the finding of either antibody alone (Senhauser *et al.*, 1963).

### Non-organ-specific Antibodies in Pernicious Anaemia

The incidence of A.N.F. was 6% in the 100 unselected pernicious anaemia patients, as against 20% in their matched controls. In the 53 pernicious anaemia patients making up the Middlesex Hospital series there were 11 with A.N.F., but these included three cases of rheumatoid arthritis, one with Sjögren's syndrome, and one with peripheral neuritis. Table VI shows the A.N.F. data for the Oxford group analysed with respect to age; there is an increasing incidence in the older patients, but no significant difference could be demonstrated between male and female subjects.

TABLE VI.—*Incidence of A.N.F. in Patients with Pernicious Anaemia and Controls Matched for Age*

| | No. Tested | Positive Reactions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | <50 (20) | | | Age-groups 50–70 (46) | | | >70 (34) | | |
| | | Wk+ | + | ++ | Wk+ | + | ++ | Wk+ | + | ++ |
| Pernicious anaemia | 100 | 0 | 0 | 0 | 1 | 0 | 1 | 4 | 0 | 0 |
| Controls | 100 | 1 | 1 | 0 | 7 | 2 | 0 | 3 | 6 | 0 |

Number of patients in each group in parentheses.

Positive A.I.C.F. reactions were obtained in 7% of 100 unselected pernicious anaemia cases and in 9% of their matched controls. Of the 53 Middlesex Hospital patients, six reacted with liver homogenates in the C.F.T.

### Gastric Antibodies in Hashimoto's Disease, Thyrotoxicosis, and Systemic Lupus Erythematosus

The second column of Table VII shows the incidence of parietal cell antibodies in the unselected pernicious anaemia group and their controls, in Hashimoto's disease, thyrotoxicosis, and S.L.E. There were 11% positive immunofluorescent reactions in the controls, 83% in pernicious anaemia, 27% in Hashimoto's disease, and 33% in thyrotoxicosis. By contrast, in S.L.E. there was only one weakly positive parietal cell reaction in 61 sera tested, though five of the patients also had thyroiditis. One S.L.E. serum produced an overall staining of all the gastric cells, possibly related to non-organ-specific antibodies.

TABLE VII.—*Incidence of Organ-specific and Non-organ-specific Antibodies in Pernicious Anaemia, Hashimoto's Disease, Thyrotoxicosis, and Systemic Lupus Erythematosus*

| | Proportion of Patients with Antibodies | | | |
|---|---|---|---|---|
| | Immunofluorescence | | | Non-organ-specific Complement Fixation (A.I.C.F.) |
| | Gastric Cytoplasm | Thyroid Cytoplasm | Nuclear (A.N.F.) | |
| Controls (matched with P.A. patients) .. | 11/100 | 13/100 | 20/100 | 9/100 |
| Unselected series P.A. .. | 83/100 | 47/100 | 6/100 | 7/100 |
| Hashimoto's disease .. | 31/113 (27%) | 574/576 (99·6%) | 8/113 (7%) | 7/113 (6%) |
| Thyrotoxicosis .. .. | 19/58 (33%) | 48/58 (83%) | 5/58 (9%) | 3/58 (5%) |
| S.L.E. without overt thyroid disease | 1/56 (2%) | 1/56 (2%) | 56/56 (100%) | 32/56 (57%) |
| S.L.E. + thyroiditis .. | 0/5 | 5/5 | 5/5 | 4/5 |

### Thyroid-function Tests

Thyroid-function tests were done in six pernicious anaemia patients, not considered originally to have thyroid disease, who were found to have high titres of thyroid antibodies (Table VIII). The tests revealed that one patient was thyrotoxic, and a repeat clinical examination confirmed this. The onset of overt toxic disease may have occurred between the two clinical examinations. In the case of one other patient, myxoedema had been previously suspected and the iodine uptake was borderline. In four patients results were within the normal range. However, since the high antibody titres suggested subclinical thyroiditis, two of these patients had repeat uptake tests after receiving dose of thyrotrophic hormone. In both the response to T.S.H. was subnormal.

## Discussion

For several reasons the immunofluorescent tests for the cytoplasmic antigens of the thyroid and stomach provide the most useful data for the demonstration of immunological interrelationships between the various diseases we have studied. With these tests 83% of pernicious anaemia patients were shown to have parietal cell antibodies, and virtually all patients with Hashimoto's disease have thyroid cytoplasmic antibodies (Table VII). Although the C.F.T. measures the same antigen-antibody systems it is less sensitive, and, unlike the immunofluorescent test, it gives positive results with sera containing non-organ-specific A.I.C.F. antibodies. The other antibodies so far demonstrated in autoimmune thyroiditis and gastritis are less reliable indices of the active disease process. Thus low titres of antibodies to the thyroid acinar colloid can occur in the absence of thyroiditis and are of uncertain significance, while, in pernicious anaemia, antibodies to intrinsic factor can be demonstrated in only 40% of cases.

Further, the thyroid antibody detected by immunofluorescence is the one responsible for the cytotoxic action of Hashimoto serum on thyroid tissue cultures (Irvine, 1962; Forbes *et al.*, 1962). It also bears a close relationship to the presence of lymphadenoid thyroid lesions in the mild subclinical forms of focal thyroiditis seen in thyrotoxic and other goitres and in middle-aged women without overt thyroid disease.

The relationship between pernicious anaemia and thyroid disease is strikingly illustrated by the high incidence of thyroid cytoplasmic antibodies in pernicious anaemia (47%) and of gastric cytoplasmic antibodies in

TABLE VIII.—*Thyroid-function Tests on Some Pernicious Anaemia Patients with Thyroid Auto-antibodies*

| Case No. | Sex and Age | Thyroid Antibodies | | Thyroid Uptake, % Dose | | | | Urinary Excretion, % Dose 6–24 hr. (15–40) | Thyroid Rate Factor ml./min. (0·04–0·12) | Red Cell $^{131}I$ Tri-iodothyronine Uptake % 100 H'crit (11·8–19·0) | Plasma Protein-bound $^{131}I$ 48 hr. % Dose/l. (0–0·57) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Without T.S.H. | | With T.S.H. | | | | | |
| | | Thyroglobulin T.R.C. Titres | Microsomal C.F.T. Titres | 4 hr. (5–40) | 24 hr. (19–53) | 4 hr. | 24 hr. | | | | |
| 1 | F 65 | 1/25,000 | 1/256 | 57·5 | 69·5 | — | — | 3·9 | 0·240 | 23·2 | 1·600 |
| 2 | F 43 | <1/5 | 1/256 | 31·0 | 44·5 | 31·0 | 55·2 | 13·7 | 0·105 | 13·1 | 0·100 |
| 3 | F 77 | 1/250,000 | 1/256 | 16·9 | 44·4 | — | — | 27·0 | 0·040 | 14·3 | 0·006 |
| 4 | F 59 | 1/250,000 | A.C. | — | 38·3 | — | — | 30·0 | 0·040 | 10·2 | — |
| 5 | F 79 | 1/250,000 | 1/16 | 20·1 | 45·8 | — | — | 19·0 | 0·110 | 12·0 | 0·006 |
| 6 | F 72 | 1/2,500 | 1/8 | 19·4 | 36·5 | 25·0 | 46·0 | 20·0 | 0·065 | — | 0·008 |

Ranges of normal values in parentheses.

Hashimoto's disease (27%) and thyrotoxicosis (33%). The results are summarized in Table VII and agree with the findings of Irvine *et al.* (1962). The abnormally high incidence of thyroid antibodies was found not only in those pernicious anaemia patients with overt thyroid disease; a high proportion with an apparently normal thyroid gave positive results. When the more significant combination of antibodies to both colloid and cytoplasm was considered, the incidence in uncomplicated pernicious anaemia was 37% compared with 8% in the controls (Table V).

Thyroid-function tests carried out on six pernicious anaemia patients with high-titre thyroid antibodies but without previously detected clinical disease of the thyroid revealed one case of mild thyrotoxicosis, one of borderline myxoedema, and a diminished thyroid reserve in two other patients. Further studies of this nature should reveal the true incidence of thyroid dysfunction in pernicious anaemia. Preliminary studies of gastric acidity in 45 patients with Hashimoto's disease have shown, by the tubeless gastric acid test, that half of them had achlorhydria, but the incidence of gastric antibodies was of the same order in the acid-producers and non-producers.

### Organ-specificity

The antibody patterns in thyroid disease and in pernicious anaemia are remarkable for the organ-specificity displayed. By contrast, in S.L.E. there is a marked lack of organ-specificity; the serum of only one patient reacted specifically with gastric mucosa and only one reacted with thyroid tissue, out of 56 without associated thyroid disease, while all were positive in the A.N.F. test and 57% showed A.I.C.F. antibodies (Table VII). Conversely, the incidence of A.N.F. and A.I.C.F. in thyroid and pernicious anaemia patients is certainly not higher than in normal controls. The control series referred to in Table VII is matched for age and sex with the unselected pernicious anaemia group and shows an unexpectedly high incidence (20%) of A.N.F., which appears to be due to the advanced age of some of the subjects tested and the inclusion of weak reactions.

Other autoimmune diseases are likely to show an overlap with thyroiditis and gastritis. Recent work (Anderson *et al.*, 1957; Blizzard *et al.*, 1962) shows that atrophic adrenalitis has an autoimmune component, and it has been known for many years that patients with Addison's disease have an increased incidence of focal thyroiditis. The serological overlap between thyroiditis and Sjögren's syndrome (Bloch *et al.*, 1960; Anderson *et al.*, 1961), and also that between discoid L.E. and thyroid autoimmunity (Shrank and Doniach, 1963), have been investigated. In both diseases a high incidence of thyroid antibodies has been found. These two conditions occupy an intermediate position in the spectrum of autoimmune disorders, since they present a mixture of organ-specific lesions characterized by lymphoid infiltration and at the same time are associated with a high incidence of A.N.F. and other manifestations related to S.L.E.

The frequent association of antibodies to thyroid, stomach, and other organ-specific antigens has many important theoretical implications. It was previously suggested that owing to the common embryological origin of the thyroid and stomach from the primitive fore-gut there may be a common auto-antigen in the two organs. For example, there is evidence that the iodide-concentrating mechanism of the thyroid, which also operates in the salivary glands and in the stomach mucosa, is mediated by common enzyme systems in the three organs, since iodide concentration was completely deficient in all three sites in a patient with a congenital metabolic error involving this function (Stanbury and Chapman, 1960). However, our present studies (Taylor *et al.*, 1962) and those of other authors (Irvine *et al.*, 1962) have shown that the gastric parietal cell and thyroid particulate antigens are quite distinct immunologically, though they have biochemical properties in common and both may be cytoplasmic precursors of organ-specific secretions.

It was previously postulated that organ-specific antigens such as those of the thyroid and other organs are isolated from the lympho-reticular system and so fail to establish immunological tolerance in the neonatal period. The microsomal antigen of the thyroid appears to be isolated in this way, and until recently thyroglobulin was thought to be similarly secluded. The finding of iodinated protein in the thyroid lymphatics (Dobyns and Hirsch, 1956) and the evidence presented by Hjort and Pedersen (1962) that thyroglobulin appears in the human circulation at birth suggest that its confinement within the thyroid is not complete. Thus some degree of immunological unresponsiveness to this protein may persist normally into adult life. However, in the human it seems that sensitization to the cellular microsomal antigen rather than thyroglobulin may be more intimately related to the development of thyroiditis lesions. The particulate organ-specific antigens are not soluble and so probably do not reach the circulation. Thus immunological tolerance to them may not be established unless contact with immunologically competent cells occurs—for instance, as a result of phagocytosis of dead cells, which is a feature of normal cellular turnover. Even then the activity of the lysosomal systems may destroy the antigenic determinants of cellular proteins before ingestion by phagocytes. If self-recognition is achieved by these mechanisms it may be less well established for specialized cells such as those of the brain, thyroid, and possibly parietal cells, which are not frequently replaced, than is the case for the components of, say, circulating blood, which are present in excess.

The finding of a frequent association of organ-specific auto-antibodies in the same individual would be consistent with an alteration in the lympho-reticular system affecting body constituents to which there was incomplete immunological tolerance, or with an increased contact between the organ-specific antigens and the lymphoid cells (either by leakage from the organ or through penetration of cytoplasm by the white cells) as a result, perhaps, of the action of a widely distributed virus, a circulating "tissue permeability factor," or a structural defect in the cellular anatomy. The existence of this serological overlap does not support the idea that autoimmunization occurs as a result of alterations in molecular structure of these auto-antigens, since one would have to postulate alterations of the antigens in several organs.

Whatever the mechanisms underlying the development of organ-specific immunity, it is clear that they must be of a different nature from those implicated in S.L.E. and similar disorders where the wide-spectrum auto-antibody pattern is clearly non-organ-specific. High titres of A.N.F., and of blood-cell and A.I.C.F. antibodies are commonly present in S.L.E., whereas organ-specific thyroid and gastric antibodies are not found in greater incidence than in healthy controls, and

are present only in low titre. This contrasts strikingly with Hashimoto's disease and pernicious anaemia, where organ-specific antibodies predominate and the incidence of A.N.F. and A.I.C.F. antibodies is low. Thus in S.L.E. there appears to be a central failure of the mechanisms responsible for the maintenance of immunological unresponsiveness to normally circulating body constituents, while these particular mechanisms seem to be largely unimpaired in the organ-specific disorders.

Our understanding of the nature of these abnormalities may be increased by study of the relatives of these patients, since both organ-specific and non-organ-specific types of autoimmune disorder appear to have a strong familial component. Thus the relatives of patients with thyroiditis have a high incidence of thyroid disease, particularly of thyroiditis and thyrotoxicosis. Pernicious anaemia is also familial. On the other hand, relatives of S.L.E. patients appear to have abnormalities of $\gamma$-globulin synthesis and a high incidence of A.N.F. Evidence is now accumulating which indicates an overlap between these two groups of disease greater than might be expected by chance (Hijmans *et al.*, 1961; Buchanan *et al.*, 1961), and, further, in diseases such as Sjögren's syndrome, discoid lupus, ulcerative colitis, and perhaps rheumatoid arthritis there is a mixture of organ-specific lesions with a non-organ-specific antibody pattern. Detailed analysis of the occurrence of autoimmune phenomena in these families should help to decide whether the genetic disturbance responsible for the two extremes of the autoimmune spectrum involves defects in two distinct compartments of the self-recognition system, one perhaps connected with the thymus and its derivative cells and the other associated with the reticulo-endothelial system (Waksman *et al.*, 1962), or whether there is a disturbance of antigen accessibility underlying the development of the organ-specific diseases.

## Summary

Because of the clinical association of thyroid diseases and pernicious anaemia the incidence has been determined of auto-antibodies to thyroid gland and stomach in patients with pernicious anaemia and healthy controls matched for age and sex, in Hashimoto's disease and thyrotoxicosis, and also in patients with systemic lupus erythematosus (S.L.E.).

The incidence of thyroid antibodies was significantly higher in pernicious anaemia patients without overt thyroid disease than in normal controls, particularly with regard to the microsomal antibody (45% against 13%), though not as high as in the thyroid diseases studied.

Gastric cytoplasmic antibodies, which were found in 83% of patients with pernicious anaemia, were detected in 27% of patients with Hashimoto's disease and 33% of patients with thyrotoxicosis.

The incidence of antinuclear factors (A.N.F.) in pernicious anaemia was lower than in the controls. No difference was demonstrated in the incidence of A.I.C.F., which was low in the two groups.

In S.L.E. the incidence of both thyroid and gastric cytoplasmic antibodies was lower than in the controls, whereas the incidence of A.N.F. was 100% and of A.I.C.F. over 50%.

Tests of thyroid function in pernicious anaemia patients with high thyroid antibody titres but without clinical evidence of thyroid disease revealed evidence of subclinical thyroiditis.

The findings in pernicious anaemia and thyroid disease are contrasted with those in S.L.E. and their significance is discussed in the light of present immunological concepts.

ADDENDUM.—Since this paper was submitted for publication two reports by Markson and Moore (1962a, 1962b) have appeared, dealing with auto-antibodies to stomach and thyroid in pernicious anaemia patients. Our findings broadly agree with the data presented by these authors.

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