Materials and Methods

**Patients.**—Sera from 287 patients with various autoimmune endocrine disorders were studied (table I). Any patient having more than one clinically diagnosed endocrine disease—e.g., diabetes mellitus and myxedema or Hashimoto's disease and pernicious anemia, &c., was classed as "polyendocrine". In all the other categories there was one overt condition and no account was taken of subclinical involvement in cases with autoantibodies to other glands. Patients with thyrotoxicosis, myxedema, or Hashimoto's goitre were described as having thyroid diseases. The 12 hypogonadal patients included cases of infertility or amenorrhoea of uncertain origin. In the 11 patients classed under "other diseases", one man had diabetes insipidus and rheumatoid arthritis. There were 3 cases of Sjögren's syndrome, one with chronic candidiasis, 1 of Raynaud's phenomenon, and 5 subjects with unrelated conditions.

**Anti-hormone sera.**—These were raised in rabbits or guinea-pigs for radiimmunoassay work, and their specificity was deemed satisfactory. Anti-growth-hormone (H.G.H.) and anti-adrenocorticotrophin (A.C.T.H.) were obtained from Burroughs Wellcome. Anti-prolactin, anti-luteinising-hormone (L.H.), and anti-follicle-stimulating-hormone (F.S.H.) were prepared at St. Bartholomew's Hospital and anti-thyroid-stimulating-hormone (T.S.H.) was made in the Nuclear Medicine Department, Middlesex Hospital. All these sera were used at a dilution of 1/10, except the anti-prolactin which could be diluted to 1/100 for immunofluorescence.

**Pituitary glands and other tissue substrates.**—Three fresh glands were obtained at hypophysectomy for advanced carcinoma of the breast in patients who had multiple metastases and had received extensive hormone therapy for some years including stilboestrol and prednisone at the time of hypophysectomy. The 3 glands were found suitable as substrates for the antibodies. Histologically they contained an increased proportion of acidophil cells which could be shown to include both prolactin and growth-hormone producing cells, while basophil cells seemed to be reduced in number. The prolactin cells were larger than those usually seen in pituitary tissue obtained at necropsy. Because the glands were removed by suction in transnasal operations they were received in the form of small fragments from different portions of the gland. Each fragment was carefully separated from blood-clots and snap-frozen in isopentane surrounded by solid carbon dioxide in acetone, and then stored at −70°C. 5 μm. unfixed cryostat sections of each fragment were tested against a known positive serum before being used for unknown sera, to ensure the presence of reacting cells in the block.

Six normal post-mortem pituitary glands were examined. In addition, two normal rat pituitaries and one from a lactating rat were tested as well as one bovine pituitary.
In all these cases the entire pituitary gland was sectioned transversely to include all parts of the anterior lobe.

The sera were also tested on human post-mortem adrenal and blood-group O pancreas, a hyperplastic parathyroid gland, thyrotoxic thyroid, and normal stomach obtained at operation, together with fresh rat testis, kidney, and liver.

Serological methods.—The sera were tested undiluted except for non-organ-specific antibodies which were detected with dilutions starting at 1/10. The usual immunofluorescence sandwich technique was used with fluorescein isothiocyanate (F.I.T.C.) conjugates of anti-human Fab, IgG, IgA, IgM, and C3 (Nordic). Titres were established by repeating the tests to end-point dilutions. All other antibodies were measured as described in the W.H.O. manual for autoimmune serology.

Double-staining technique.—To identify the type of pituitary cells reacting with the patients’ sera, it was necessary to use a double-sandwich technique on the same section. The appropriate anti-hormone antibody was applied for forty minutes followed by goat-anti-rabbit-γ-globulin conjugated with rhodamine (red immunofluorescence). The patient’s serum was then applied, followed by sheep-anti-human-Ig-F.I.T.C. conjugate (green immunofluorescence). The sections were washed for fifteen minutes between each stage in 3 changes of Coons’ buffer without agitation. As a control in each experiment the reverse procedure was used—i.e., patient’s serum and F.I.T.C. conjugate were applied to the section first. Essential controls also included sections stained with one or the other serum and both conjugates, to detect non-specific inter-species reactions.

Double-exposure photographs were taken with automatic camera attached to Leitz Ortholux microscope fitted with epi-illumination. For F.I.T.C., BG38 and 2KP490 excitation filters were used, with an S525 suppression filter. For rhodamine, BG38 and S546 excitation filters were used with a K570 suppression filter. When the same cells were stained by both conjugates they appeared orange with suppression filter K510. The green photograph was taken first, followed by the mixed, then the red on the same field.

Nature of Pituitary Antibodies

Of the 287 sera tested, 19 gave a diffuse, finely granular cytoplasmic immunofluorescence on certain cells of the anterior pituitary in the glands obtained at operation for carcinoma of the breast. The distribution of positive cells was the same for all these sera (fig. 1). Some cells were more brightly stained than others, and many other cells (in some parts of the section) appeared unstimulated. With the 268 negative sera, the pituitary “colloid” stained non-specifically, and inter glandular connective-tissue fibres appeared slightly green. A pink to orange auto-

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Fig. 1—Unfixed cryostat section of anterior pituitary lobe obtained at hypophysectomy in a patient with metastasizing breast cancer and containing hypertrophied prolactin cells.

This section was treated with serum from a 75-year-old woman having a combination of autoimmune thyroiditis, Addison’s disease, and diabetes mellitus, followed by sheep-anti-human-Fab conjugated with F.I.T.C. There is finely granular diffuse cytoplasmic immunofluorescence. By double-sandwich technique with 2 fluorochromes it was possible to demonstrate that these were prolactin-secreting cells. Reduced to half from 4×50.

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Fig. 2—Adjoining section of adenohypophysis treated with normal serum and anti-Ig conjugate showing no cytoplasmic staining.

The bright dots are pink lipofuscin granules.

fluorescence due to lipofuscin granules was seen in some cells, but did not interfere with the fluorochromes (fig. 2). With four of the “strongest” sera, using the double-sandwich technique, it was possible to demonstrate that H.G.H., A.C.T.H., T.S.H., L.H., and F.S.H. cells remained red and were distinct from the green cells stained by the patient’s sera. When antiprolactin serum was applied, all the stained cells were orange with filter K510, suggesting that they were stained by both the anti-hormone and the patient’s antibodies. This could be confirmed by double-exposure photography in any one field using appropriate filters for each fluorescent dye. With the post-mortem pituitary glands, strongly positive sera stained occasional small oval cells, consistent with the low frequency and resting state of prolactin-secreting cells in these glands, but the results were difficult to interpret, since normal sera also stained occasional cells, albeit producing a smooth non-granular pattern. Experiments with animal pituitaries were inconclusive owing to substantial non-specific fluorescence and the sparsity of prolactin cells. The pituitary from a lactating rat proved equally unsuitable, but more experiments are required.

The titres of prolactin-cell antibodies were similar to those generally observed when adrenal, pancreatic, or parathyroid glands were tested against their respective organ-specific autoantibodies, and varied from a weak reaction with undiluted serum to about 1:80.
When the positive cases were retested with monospecific antiglobulins, all 19 sera contained IgM antibodies, 17 were positive with anti-IgG, and 14 with anti-IgA. In 10 of the sera, IgA antibodies gave the brightest immunofluorescence. Complement-fixing activity was demonstrated in 6 sera tested with anti-C3 conjugate. The antibodies could still be detected after storage at -20°C for up to five years. In 7 cases, two to five specimens of serum had been obtained from the patient at various intervals. The antibodies were detectable in each of these, although the intensity of the fluorescence varied from time to time in the same patient.

**Fixation Experiments**

The tissue antigen involved in the reaction with prolactin-cell antibodies was resistant to fixation for 10 minutes at room temperature in acetone, 100% methyl alcohol, and 1% formaldehyde freshly prepared from paraformaldehyde, while thirty minutes' fixation greatly reduced the staining. It also resisted three minutes' fixation in 0.2% glutaraldehyde but was destroyed after ten minutes in this fixative.

**Clinical Correlations**

The clinical diagnoses and full serological results on the 19 patients with prolactin-cell antibodies are shown in table II. There were 11 females varying in age from twelve to seventy-five years, and 8 males aged eleven to sixty-nine years. 10 of the patients (8F, 2M) had more than one clinically diagnosed endocrine disease, while in 9 patients (3F, 6M) a single endocrine disease had been diagnosed (except for 2 patients who had an associated collagen disorder). No patient in this series had symptoms or signs of hypopituitarism. 12 cases of idiopathic hypoparathyroidism (4 polyendocrine) were included in this study and 4 had pituitary antibodies, although sera from only 2 of the 12 reacted with human parathyroid gland. There were autoantibodies to other endocrine glands in all 19 patients whose sera reacted positively with the pituitary gland, probably because of preselection of the material. Thus 15 had one or both thyroid antibodies, 15 reacted with adrenal tissue,

<table>
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<th>Adrenal cortex</th>
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<th>Parathyroid</th>
<th>Thyroid</th>
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D.M. = diabetes mellitus; P.A. = pernicious anemia; micro = microsomal antibodies; I.F.L. = immunofluorescence; H.A. = Hereditum hereditary agglutination test; Tgb = tanned red blood-cell agglutination test for thyroglobulin antibodies; G.P.C. = gastric parietal cells; I.F. = intrinsic factor; A.N.A. = nuclear antibodies; S.M.A. = smooth muscle fluorescent antibodies at 1/10; Latex Fitz = rheumatoid factor; s.C.A.T. = sheep red-cell agglutination titre. + = positive only with undiluted serum; ++ = positive, not titrated. - = test negative.
8 of these also showing positive Leydig-cells typical of the second adrenal antigen common to steroid-producing cells in ovary, testis, and placenta. 8 patients reacted with pancreatic islet cells and 4 with parathyroid gland (table III). Although there were more males with a single endocrine diagnosis, there does not appear to be any significant difference between these cases and the polyendocrine patients in the frequency and titres of various antibodies. 7 patients with adrenal antibodies showed no evidence of Addison's disease, and only 1 of 4 cases with parathyroid immunofluorescence had evidence of hypoparathyroidism. Diabetes mellitus was present in 5 of 8 patients with islet-cell antibodies. Subclinical lesions with antibodies are commonplace in thyroiditis and gastritis, and in this series 8 of 15 cases with thyroid antibodies had overt thyroid disease and only 3 of 9 with gastric autoimmune had pernicious anemia.

Non-organ-specific antibodies were uncommon and of low titre; the 2 patients with rheumatoid arthritis were both seropositive. 1 patient, a man aged forty-seven (case 12), is of special interest in that he developed diabetes insipidus and rheumatoid arthritis affecting his ankles and wrists at the age of forty. There is no evidence of a hypophalamic tumour, but in addition to rheumatoid factors (R.F) he has high titres of antibodies to adrenal cortex, thyroid, and stomach, and only subclinical defects in these organs.

Discussion

The concept of an autoimmune type of hypopituitarism was first suggested by Goudie and Pinkerton when they found destructive lesions of the anterior pituitary with heavy lymphoid infiltration at necropsy in a young woman who also had thyroiditis and adrenal atrophy. There are isolated reports of pituitary lymphoid focus in patients with idiopathic Addison's disease and in other post-mortem studies. Attempts have also been made to produce a hypophysis in animals by injecting them with pituitary extracts in Freund adjuvant. However, there are no previous descriptions of pituitary antibodies either in cases of hypopituitarism or in other disorders.

The detection of pituitary antibodies proved quite difficult. It was perhaps fortunate that the present attempts were successful using suspensions from a case of breast cancer and that the sera applied to this gland were from patients who were already known to have autoimmunity involving other endocrine glands. The pituitary immunofluorescence obtained with the serum of case 1 was so striking (fig. 1) that it could not be dismissed as a non-specific reaction. We were further encouraged when we found that 3 other sera out of some 40 tested on this gland showed positive staining of a similar pattern. Numerous blocks from post-mortem pituitaries were then examined with these positive sera, but the efforts proved unrewarding owing to the low number of reacting cells and their small size. Animal glands were equally useless for extensive screening.

When a second hypophysectomy specimen from a breast-cancer patient gave clear-cut results and sufficient tissue was available, it was possible to test a larger number of sera and to identify the cell-type involved in the reaction. This was made possible by the availability of highly purified animal antisera to each of the six pituitary hormones. With the double immunofluorescence sandwich technique it was demonstrated that five hormone antisera stained different cells from those reacting with the patients' sera, and only anti-prolactin produced the same pattern of staining. The fact that the prolactin cells were doubly stained suggests that the autoantibodies were not directed against the hormone itself. By analogy with other organ-specific antigen-antibody systems, the autoantibodies probably reacted with cytoplasmic organelles involved in the synthesis or delivery of the hormone. Studies are in progress to demonstrate the exact location of the antigen by immunoelectron microscopy with peroxidase conjugates.

The clinical importance of prolactin-cell antibodies is at present obscure. Accurate radioimmunoassays for this hormone are new. Apart from its role in reproduction and lactation, prolactin is thought to have other undefined metabolic functions in man. Prolactin deficiency states have not been described as yet, but by analogy with myxedema, pernicious anemia, and Addison's disease they might be expected to produce gradual autoimmune destruction of the prolactin cells. Of the patients in this series had T.S.H. releasing factor tests to demonstrate the simultaneous discharge of T.S.H. and prolactin known to occur after injection of the hypophalamic tripeptide.

The results in 2 patients gave flat curves, but the other 7 responded normally, despite replacement therapy with thyroxine insulin or corticosteroids.

Prolactin-cell antibodies often seem to be associated with idiopathic hypoparathyroidism, since 4 of the 12 cases included in this study showed positive immunofluorescence on these cells, and in two families with several cases of autoimmune polyendocrinopathy being investigated immunologically at the time of writing, all 5 cases of idiopathic hypoparathyroidism had prolactin-cell antibodies, although only 1 of them had serum that reacted with parathyroid-gland tissue. Normal subjects have not yet been examined systematically by immunofluorescence techniques on pituitaries from patients with breast cancer, but the large number of negative cases in our highly selected material suggests that prolactin autoantibody will be uncommon in the general population.

The finding of autoimmunity to a single pituitary cell-type opens up interesting possibilities for future studies, since isolated clinical defects involving the other five pituitary hormones have been reported on many occasions and are known to occur in adults, which suggests that enzyme deficiencies are not the cause. Although in many cases the defect has been traced to the hypophalamic, the lesion could be in the pituitary in some patients. We are at present investigating 3 sera which seem to react with a different cell-type in pituitary tissue from patients with breast cancer, so there is hope of finding sera that will react with thyrotrophs or somatotrophs, for instance. For this it may be necessary to select patients with isolated T.S.H. or H.G.H. defects and to apply their sera to pituitary glands where the appropriate cells are hypertrophied. We have attempted immuno-
fluorescence test on a pituitary gland removed because of Cushings syndrome. We found that normal sera often reacted with A.C.T.H.-producing cells, and the significance of this is at present unknown.

Much further work is required to obtain a real understanding of pituitary autoimmunity, but the existence of such conditions is suggested in the present study.

We thank Mr. R. A. Williams for the hypophysectomy specimens, Dr. J. D. N. Nabarro, Prof. Michael Besser, and Dr. Susan van Noorden for assistance with problems of endocrinology; Prof. I. M. Raitt for his valuable support; Dr. S. Franks and Dr. R. Edwards for hormone antiserum; Mr. Granville Swann and Mrs. Marlene Swans for technical help; and Miss Hilary Fischler for preparing the manuscript. G. F. B. has a British Council scholarship and A. F. has a Medical Research Council fellowship. Work in the department of immunology is supported by grants from the World Health Organisation and the Medical Research Council.

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HYPERSECRETION AND LENGTH OF HISTORY IN DUODENAL ULCERATION

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Summary Maximal gastric secretion, measured by the histamine-infusion test, was, as expected, significantly greater in a group of 81 patients with duodenal ulcer than in a group of 72 controls. After standardising for the effect of stature on maximal secretion, only 24 of the patients were found to be true hypersecretors. However, maximal secretion in the ulcer group increased with length of history of symptoms, and extrapolation back to zero length of history suggested that there was no significant hypersecretion at that time. These facts support the hypothesis that it is the presence of the ulcer that leads to hypersecretion rather than the converse. Possible mechanisms involved are chronic ingestion of antacids to counter dyspepsia, or gastric distension due to pylorospasm.

Introduction

Patients with duodenal ulceration tend to have a maximal gastric secretory capacity that exceeds the capacity of a control group. This finding, together with the well-known observation that reduction in gastric secretory capacity (e.g., by partial gastrectomy or vagotomy) usually results in the healing of duodenal ulceration, has led to general acceptance that gastric hypersecretion accounts at least partly for duodenal ulceration.

Yet many patients with a duodenal ulcer have a maximal gastric secretion within the normal range. Describing the augmented histamine test, Kay stated "There is clearly no place for hyperacidity as an aid to diagnosis". Therefore, gastric hypersecretion cannot be the sole cause of duodenal ulceration.

Hassan and Hobsley, comparing maximal secretion in normal subjects and duodenal-ulcer patients, drew attention to the importance of correcting for pyloric losses and stature. The decision whether or not a patient with duodenal ulceration is a hyper-secretor may depend upon whether these factors are heeded or ignored. Hassan and Hobsley noted that maximal secretion in control adult subjects appeared to diminish as age increased, but surprisingly that in duodenal-ulcer patients hypersecretion became more prominent in those with a longer rather than a shorter history. Since older patients might be expected to have longer histories, these findings appear contradictory and require elucidation. We examine here the problem in a larger series of subjects, using statistical techniques that permit discrimination between the effects of these various factors.

Clinical Material

Maximal gastric secretion was measured in 72 normal subjects and in 87 patients with duodenal ulcer. The normal group included symptomless volunteers, and hospital patients who had no symptoms that could reasonably be linked with peptic ulceration and who volunteered to be investigated. Some of the studies described by Hassan and Hobsley are included in this series.

Methods

Height, weight, sex, and clinical details were recorded, at 9 a.m., before the secretion study began. Food, drink, tobacco, and all drugs known to affect gastric secretion were withheld from the preceding midnight. The lean body mass (L.B.M.) of each subject was calculated using the formula described by Hume. The subject lay semi-recumbent, and swallowed a specially prepared two-lumen nasogastric tube. The tip of the tube was confirmed to be in correct position in the stomach by the water-recovery test. Gastric contents were aspirated continuously, and phenol-red was instilled into the stomach as described by Hobsley and Silen. The rate of instillation of phenol-red, 30 g per litre solution, was 10-4 ml per hour. An intravenous infusion of histamine acid phosphate, 0.13 nmol per kg per hour (0.04 mg per kg per hour), was begun.

After a plateau of maximal gastric secretion was reached, gastric juice was aspirated continuously and collected in 10-minute samples for at least four samples. For each sample, the volume was measured to the nearest 1 ml, and it was then filtered through Whatman's no. 1