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HUMAN AUTO-IMMUNE THYROIDITIS: CLINICAL STUDIES

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In a recent paper (Roitt and Doniach, 1958) serological studies were reported in 644 patients with various thyroid diseases tested for circulating thyroid auto-antibodies. The finding of these antibodies in patients with chronic thyroiditis (Roitt, Doniach, Campbell, and Hudson, 1956; Witebsky, Terplan, Paine, and Egan, 1957) was confirmed and the subject considerably extended by workers in other centres (Trotter, Belyavin, and Waddams, 1957, Goudie, Anderson, Gray, Clark, Murray, and McNicol, 1957; White, 1957; Owen and Smart 1958; Anderson, Goudie, and Gray, 1959a, 1959b; Goudie, Anderson, and Gray, 1959; Belyavin and Trotter, 1959; Blizzard, Hamwi, Skillman, and Wheeler, 1959; Cline, Selenkow, and Brooke, 1959; Beierwaltes Dodson, and Wheeler, 1959) and by our own studies; and the subject was reviewed by Owen (1958) and by Mahaux (1959).

Since 1956 we have tested for antibodies every thyroid patient attending a surgical clinic at the Middlesex Hospital and have also been fortunate in obtaining for study sera from cases in many other hospitals. This paper is intended to summarize the clinical experience gained in the course of these studies and to emphasize several features of auto-immunizing thyroiditis which became apparent as a result of diagnosing early and unusual cases of the disease, and of following them up with repeated estimations of auto-antibody levels and other thyroid-function tests without surgical intervention.

The connexions of auto-immunizing thyroiditis with primary myxoedema and thyrotoxicosis, and its relationships with subacute thyroiditis (de Quervain's disease), recently shown to be of viral aetiology (Eylan, Zmucky, and Sheba, 1957), have been studied. It appears that some patients with virus thyroiditis also have auto-antibodies to thyroid antigens, but this very rarely leads to progressive destruction of the gland. Evidence is presented to show that some cases of auto-immune thyroiditis having a subacute onset are difficult to distinguish clinically from de Quervain's disease. Furthermore, auto-immune thyroiditis can sometimes assume a fluctuating course in which subacute episodes alternate with long periods of quiescence. Though in most patients the auto-antibodies are strictly organ-specific, there is evidence in rare cases of a more widespread disturbance of immunological responses of the type seen in systemic lupus erythematosus.

Methods

Immunological methods used for the detection of auto-antibodies included in all cases the tanned red-cell agglutination test (T.R.C.) and precipitin test in agar (P.P.) for antibodies to thyroglobulin, and the complement-fixation test (C.F.T.) using extracts of thyrotoxic glands to detect antibodies to the "microsomal" auto-antigen of thyroid cells. In addition, patients giving positive results with thyrotoxic extracts were also tested by the C.F.T. against extracts of human liver, kidney, suprarenal, normal thyroid, and in some instances lacrimal and salivary glands.

All thyroid patients were investigated with ^{131}I tracer tests, which included the 24-hour neck uptake curve and urinary excretion, 48-hour plasma content and its protein-bound fraction, and the topographical survey of isotope distribution over the thyroid. In cases showing a raised 24-hour uptake in the absence of overt hyperthyroidism a triiodothyronine (T_3) suppression test (Werner and Spooner, 1955) is now performed to exclude true thyrotoxicosis. For this, 120 μg of T_3 is given daily in divided doses for 10 days, followed by a repeat of the uptake test. The biological half-life of isotope retention was measured in isolated cases, and the perchlorate test (Morgans and Trotter, 1957) and TSH stimulation tests were used in some patients. The basal metabolic rate (B.M.R.) was measured in all patients, using the Robertson and Reid (1952) standard, and the serum cholesterol was estimated; the P.B.^{127}I is now included. The sero-flocculation tests, thymol and zinc sulphate turbidities, colloidal gold test, and paper electrophoresis were carried out in most cases where antibodies of appreciable titre were detected or where the case was suggestive of lymphadenoid goitre on clinical grounds. Other liver-function tests, including serum bilirubin, alkaline phosphatase, and bromsulphalein retention tests, were done when associated liver disease was suspected. The results of tests are given in illustrative cases, but are not discussed in detail in the present paper.

Clinical Observations

Before the description of case reports and their discussion it is relevant to summarize the overall results obtained with serological methods in the principal thyroid diseases. These are shown in Fig. 1; antibody levels have been classed as low, medium, and high in relation to titres found in untreated Hashimoto's disease where the incidence of positive results was 98%, and the highest precipitin level found so far was 18.9 mg. of antibody protein/ml. of serum (cf. Mahaux and Pirart, 1959) with more usual levels of up to 7 mg./ml. In primary myxoedema 83% of cases had antibodies, while the corresponding figures in thyrotoxicosis, non-toxic

nodular colloid goitre, and thyroid cancer were 67%, 33%, and 29% respectively. Titres were much lower in the last three conditions, though the frequency of positive results corresponds well with the known incidence of patchy lymphoid infiltration in these goitres. A control group of 195 unselected hospital patients without thyroid disease gave negative results

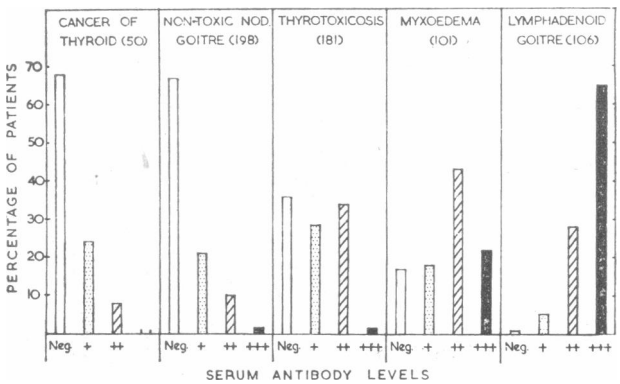

FIG. 1.—Incidence of circulating thyroid auto-antibodies in thyroid diseases, tested by the precipitin reaction in agar (P.P.), tanned red-cell agglutination (T.R.C.), and complement-fixation test (C.F.T.). Figures in parentheses indicate number of patients tested in each group. The levels of serum antibodies were divided into weak, medium, and high titres as follows: *Weak* (+) T.R.C. titres 5–250 and/or C.F.T. titres less than 1/20 serum dilution, using two minimum haemolytic doses of complement (2M.H.D.) for the test. (*Medium* (++) T.R.C. titres 2,500–25,000 and/or C.F.T. titres of 20 or over. *High* (+++) positive precipitins; in these cases the T.R.C. titre was usually between 25,000 and over 2,500,000 (serum dilutions in steps of 10 made with a single pipette), and complement-fixing antibodies were also present.

in the C.F.T. by the method used for the thyroid patients, while 6% of 148 control subjects had positive T.R.C. tests with titres varying between 5 and 250.

Illustrative Cases of Classical Hashimoto's Disease

Case 1

Housewife aged 65. Small symptomless goitre for at least 10 years. After an attack of influenza complicated by pneumonia in October 1957, the goitre became tender and larger. Since then she felt tired, was breathless on exertion, and felt the cold more acutely. In April, 1958, she had an attack of bronchitis with purulent sputum and fever lasting one week. The goitre enlarged rapidly to twice its previous size, producing a choking sensation. Continuous tiredness and increasing breathlessness brought her to hospital. There were no previous illnesses and no history of rheumatic fever. Since the attack of pneumonia she has suffered from asthma and has had some urticaria. Family history: the first of her three daughters was born deaf and had a non-toxic goitre.

On examination: Tired appearance without obvious myxoedema. Firm diffuse goitre, estimated weight 120 g.; bosselated surface, globular lateral lobes, larger on the right, with moderately enlarged isthmus. P.R. 60, B.P. 110/65. Harsh systolic murmur at apex, conducted to axilla, probably due to mitral incompetence. No ankle oedema; liver and spleen not palpable.

Investigations.—B.M.R. +6%, serum cholesterol 260 mg./100 ml. ^{131}I uptake 50%, urinary excretion 37%/24 hours, 48-hour plasma activity 0.43%, P.B.^{131}I 0.23% per litre. Topographical survey showed symmetrical uptake. Total serum proteins 8.6 g./100 ml. (albumin 3.6 g., globulin 5 g.). On electrophoresis raised γ-globulins, thymol turbidity 24 units, colloidal gold 5 units, zinc sulphate 33 units. E.S.R. 22 mm. (1 hour Westergren), Hb 81%, W.B.C. 4,900/c.mm., E.C.G. normal, chest *x*-ray film showed left ventricular enlargement. *Thyroid auto-antibodies:*—Precipitin test strongly positive. T.R.C. titre ⩾ 2½ million. C.F.T. titre 128 with thyrotoxic thyroid extract, negative with normal thyroid, liver, kidney, suprarenal, and brain.

Treatment.—L-thyroxine 0.3 mg. daily. Feels and looks better; less breathless and has more energy. Goitre reduced in size to estimated 50 g. after one year; softer but still finely bosselated.

Case 2

Housewife aged 61 first noticed goitre soon after menopause at age 52; gradually increased in size since, no symptoms except gain in weight from 10 st. to 12 st. (63.5 to 76.2 kg.); no tiredness or cold sensitivity. No family history of goitre and no previous diseases.

On examination: Obese with placid expression. P.R. 82, B.P. 140/90. Large firm symmetrical goitre, estimated weight 150 g. Liver and spleen not palpable.

Investigations.—B.M.R. +7%; ^{131}I uptake 24%, urinary excretion 48%/24 hours. Topography: symmetrical thyroid-shaped distribution. Serum proteins 8.5 g./100 ml. (albumin 4.3 g., globulin 4.2 g.); γ-globulins markedly increased; thymol 15 units, zinc 32 units, gold 5 units. *Thyroid auto-antibodies:*—T.R.C. titre only 250, but atypical precipitin with fluffy edges appeared after six days in agar. C.F.T. titre 1024 with thyrotoxic gland. No complement fixation with other organs. Thyroid needle biopsy (taken by R. Turner-Warwick, report by A. D. Thomson): "Sections from right and left lobes show a heavy lymphoid infiltration and altered eosinophil thyroid epithelium characteristic of lymphadenoid goitre."

Treatment.—Thyroid extract 3 gr. (200 mg.) daily. Felt no different but lost over a stone (6.4 kg.) in weight, which suggests very early thyroid deficiency. The goitre decreased in size very slowly after one and a half years. C.F.T. titre reduced to 256, but still has the same atypical precipitin. Thymol and zinc turbidities down to 5 units and 15 units respectively.

Case 3

Housewife aged 48. Goitre for two and a half years and increasing tiredness. Gained 1 st. (6.4 kg.) in weight; skin dry and scaly; feels cold; falls asleep in daytime. Abdominal distension and flatulence; constipated. Past illnesses included only repeated attacks of quinsy, laryngitis, and tonsillitis. No rheumatism, asthma, or other allergic manifestations. No family history of goitre.

On examination: Myxoedematous facies. Firm, diffuse goitre, estimated weight 30 g. P.R. 50, P.B. 130/80. Liver and spleen not palpable.

Investigations.—B.M.R. −43%; cholesterol 650 mg./100 ml. ^{131}I uptake 8%; urinary excretion 0–24 hours 49%, 24–48 hours 25%; 48-hour plasma activity 0.72%; P.B.^{131}I 0.18%/l. (high total activity probably due to slow iodide excretion). E.S.R. 47 mm. (1 hour Westergren). Total proteins 8.2 g./100 ml. (albumin 4.6 g., globulin 3.6 g.); γ-globulins raised; thymol 18 units; zinc sulphate 27 units, colloidal gold negative. Serum bilirubin less than 0.4 mg./100 ml., alkaline phosphatase 2 units. Bromsulphalein excretion normal. E.C.G.: sinus bradycardia, small complexes, and widespread T inversion suggestive of myxoedema. *Thyroid auto-antibodies:*—T.R.C., C.F.T., and precipitin all negative on nine separate occasions. *Thyroid biopsy* (report by R. E. Cotten): Sections show scanty atrophic acini and very marked lymphocytic infiltration with follicle formation; appearances are of advanced lymphadenoid goitre.

Treatment.—Thyroid 2 gr. (130 mg.) daily. All symptoms disappeared.

Comment

The clinical features in these three patients are typical of advanced chronic thyroiditis as described by Hashimoto. The onset was insidious and apparently not preceded by thyroid infections or other diseases. All three were middle-aged women and had firm, diffuse

goitres; one was frankly myxoedematous, one was mildly hypothyroid, while the third was euthyroid; all three had raised γ-globulins and grossly abnormal flocculation values. Serologically they exemplify the various combinations of auto-antibodies usually met with. Case 1 had both precipitins and C. F. antibodies of high titre; Case 2 had mostly C.F. antibodies with a low T.R.C. titre, and her atypical precipitins may well be directed against an antigen distinct from thyroglobulin (c.f. Roitt, Campbell, and Doniach, 1958). Case 3 gave completely negative results by the three tests used, despite her raised γ-globulins. There is evidence that her serum contains antibodies demonstrable in the colloid by Coons's fluorescent antibody technique (Balfour, Doniach, and Roitt, 1959). Case 1 shows a feature which is seen not uncommonly; her goitre rapidly enlarged after each of two respiratory infections, and she shows evidence of allergic manifestations. In view of her family history of goitre with deafness (Morgans and Trotter, 1958) it is possible that she had a thyroid abnormality preceding the lymphadenoid change.

Connexion Between Lymphadenoid Goitre and Primary Myxoedema

It seems probable from results published so far that "primary" myxoedema occurring in adult life is a variant of advanced auto-immune thyroiditis and differs from Hashimoto's disease only in the fact that the thyroid undergoes atrophy instead of responding to T.S.H. stimulation by continuous regeneration with goitre formation. Myxoedema with thyroid atrophy is about three times more frequent than lymphadenoid goitre, and, though the latter can reach a size of 20 times the normal gland, small goitres are far more common and are often missed. In many patients the thyroid is not enlarged but is palpable owing to its firm consistency if specially looked for. It has not been sufficiently stressed that a goitre may be present at one stage and later regress spontaneously, giving rise to apparent primary myxoedema.

Case 4.—Woman aged 92. Admitted to Middlesex Hospital in 1896, aged 29, with a fairly large firm symmetrical goitre of four months' duration, accompanied by pressure symptoms and lassitude. She was given thyroid extract for one month with improvement, but had no further treatment, and was not seen again until 1904, when she had advanced myxoedema and no palpable thyroid. She states that her goitre gradually disappeared over several years, while she felt increasingly tired and slow. She has been on thyroid continuously for 55 years and is well. Her serum still contains a small trace of antibodies by the T.R.C. test.

Case 5.—Woman aged 31. First seen at age 27 with two and a half years' history of goitre and lassitude. On examination, clinically euthyroid; firm diffuse goitre, estimated weight 40 g. B.M.R. −7%; ^{131}I uptake 44%; plasma clearance rate at 1 hour, 40 ml./min. (upper limit of normal). Patient moved away and failed to return for treatment until four years later, when she was found to be profoundly myxoedematous and had no palpable thyroid. B.M.R. −33%, ^{131}I uptake 13%, excretion 72% in 24 hours. *Thyroid antibodies:*—Complement fixation and T.R.C. test weakly positive.

Comment

Spontaneous resolution of lymphadenoid goitre, with atrophy of the thyroid and progressive hormonal failure, probably occurs, particularly in patients who have small or moderate goitres even at the height of the gland's regenerative efforts. Large Hashimoto goitres do not as a rule regress spontaneously; sometimes they even recur after partial surgical removal when patients are not given adequate substitution therapy.

Thyroid Auto-immunity and Graves's Disease

Spontaneous regression of a goitre with ultimate myxoedema has been noted more often in thyrotoxic patients (Pasteur, 1900; Brünger, 1914; Eason, 1928), and this appears to be connected with the presence of auto-immunizing thyroiditis.

Case 6.—Woman aged 60. Presented with advanced myxoedema. On examination no palpable thyroid but some residual exophthalmos. At age 27 had thyrotoxicosis with a goitre and eye signs. Symptoms lasted for one and a half years and were treated with iodine. The goitre gradually disappeared and the patient remained euthyroid until age 54, when she began to feel tired and cold, gained 3 st. (19 kg.) in weight and had a puffy face. Symptoms increased for several years before she was seen with a B.M.R. of −27%; ^{131}I uptake 12%/24 hours. Thymol turbidity 6 units, zinc sulphate 11 units. *Thyroid antibodies:*—Weak positive complement fixation.

Case 7.—Housewife aged 37. Presented as a classical case of Hashimoto's disease with diffuse firm goitre, estimated weight 45 g., and myxoedema. In 1946 she rapidly lost weight from her normal 8 st. to 5 st. 7 lb. (50.8 to 34.9 kg.) in spite of a good appetite; felt nervous and run down and had marked trembling. The symptoms were attributed to "war strain" and "nerves," and no specific treatment was given though the illness lasted for nearly two years. Patient remained well for seven years before she developed increasing myxoedema. The goitre was small for some years, but became larger and tender after each of two respiratory infections. On investigation, B.M.R. −29%, serum cholesterol 360 mg./100 ml., P.B.I. 1.4 μg./100 ml., ^{131}I uptake 29%, excretion 37%/24 hours; 48-hour plasma activity 0.69%, protein-bound fraction 0.48%/l. Serum proteins 7.9 g./100 ml., γ-globulins slightly raised, thymol turbidity 6 units, zinc sulphate 13 units, colloidal gold 3 units. Serum bilirubin 0.6 mg./100 ml., alkaline phosphatase 3.3 K-A units. *Thyroid antibodies:*—Precipitin test strongly positive, T.R.C. titre over 2 million, C.F.T. titre 128. Goitre and symptoms responded well to thyroid extract.

Case 8.—Housewife aged 44. Presented April, 1951, with a history of five months' nervousness, loss of weight 1 st. (6.4 kg.) in spite of good appetite, breathlessness, and trembling; goitre and prominent eyes noticed for four months. B.M.R. +33%; thyrotoxicosis diagnosed, but when admitted for operation in July, 1951, her toxic symptoms had diminished, the B.M.R. had decreased to 0 without specific treatment, and the goitre had become firm. ^{131}I uptake was 41%. Lymphadenoid change was suspected, and she was discharged without operation. Myxoedema gradually developed two years later and the goitre enlarged, but all symptoms subsided with thyroid 2 gr. (130 mg.) daily. γ-Globulins were still raised to 2.7 g./100 ml.; thymol turbidity 7 units, and precipitins were strongly positive after five years' treatment with thyroid hormone.

Case 9.—Man aged 49. In this case the transition from moderate Graves's disease with a soft thyroid of approximately 45 g. to full-blown large Hashimoto goitre with progressive oedematous exophthalmos and pretibial myxoedema was observed over a period of 18 months with the appearance of increasing levels of auto-antibodies (Doniach and Hudson, 1959). A subtotal thyroidectomy was undertaken for persistent thyrotoxicosis with evidence of cardiac insufficiency and increasing pressure symptoms; the gland weighed 132 g., and was completely replaced by lymphadenoid tissue showing extensive Askenazy-cell change. The rise in thyroglobulin precipitin levels during the developing stage of the lymphadenoid process and the

abrupt decline following subtotal thyroidectomy are shown in Fig. 2. The complement-fixation titre likewise rose gradually from 64 to 512 and declined after operation. The patient's exophthalmos increased rapidly six weeks after removal of the thyroid despite treatment with thyroid extract 3 gr. 200 mg.) daily. Deep *x*-ray treatment to the orbits has not influenced the proptosis in the succeeding four months, but has improved the diplopia. The patient feels very much better than before operation; his ankle oedema and multiple extrasystoles have completely subsided.

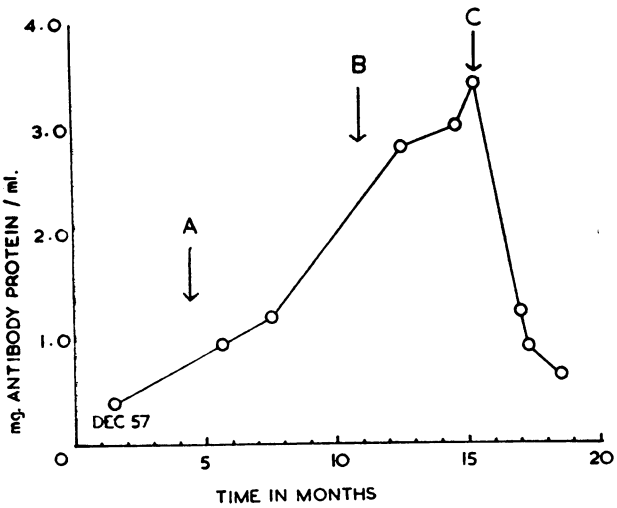

FIG. 2.—Case 9. Thyroglobulin precipitin levels. Arrows A and B indicate two febrile episodes due to upper respiratory infections, during which the goitre became tender. Following the first infection the goitre enlarged rapidly and a further exacerbation occurred after the second infection. Subtotal thyroidectomy is indicated by arrow C. Antibody levels were determined by a co-precipitation method using radioactive thyroglobulin and anti-human γ-globulin serum (Roitt and Doniach, 1959).

Comment

Several other patients in whom auto-immune thyroiditis led to remission of thyrotoxicosis and subsequent myxoedema or Hashimoto's disease have been investigated, and the above cases were chosen to illustrate the marked variations of speed and intensity with which thyroid destruction can progress. Complete invasion of the thyroid may take 30 years or may sweep through the gland in a few months. Extensive lymphadenoid change is compatible with persistence of severe hyperthyroidism owing to the functional reserves of the thyrotoxic gland.

Progressive thyroid destruction is rare when thyrotoxic patients are considered as a whole. In the great majority of cases small aggregates of lymphocytes or even patches of lymphadenoid tissue with follicle formation, disrupted acini, and Askenazy-cell change are seen in the gland, and low-titre antibodies are found in the serum but the lesions are of low-grade intensity. Even when lymphoid replacement is more extensive, the process may be quiescent and thyrotoxicosis may supervene after thyroidectomy, as shown in the case reported by Joplin and Fraser (1959). We have observed a similar case in which thyrotoxicosis developed 10 years after the removal of a non-toxic goitre showing marked focal thyroiditis. However, such instances are rare, and statistically the incidence of post-thyroidectomy myxoedema increases with the extent of lymphadenoid replacement in thyrotoxic glands (Whitesell and Black, 1949; Greene, 1953).

Auto-immune Thyroiditis with Subacute Onset

This form of lymphadenoid goitre is of particular diagnostic interest since it may resemble de Quervain's thyroiditis clinically. Unlike true virus thyroiditis, it often leads to permanent impairment of thyroid function.

Two cases of Hashimoto's disease with subacute onset were studied by us before serological tests became available (Doniach and Hudson, 1957, Cases 1 and 2). These patients (E. M. and Y. C.) developed a tender goitre within a few weeks, with neck pain radiating to the mastoid area. Their iodine uptakes were 72% and 65%/24 hours respectively 10 and 4 weeks after onset of symptoms though the patients were euthyroid clinically. This high uptake was maintained for a further three months in the first patient (E. M.), and her goitre regressed without thyroid administration, while in the second patient the iodine uptake dropped to 52% five months after onset. Both patients had a low metabolic rate, a markedly raised E.S.R., and high γ-globulins. The diagnosis of Hashimoto's disease was confirmed histologically.

Since then several more rapidly developing cases of auto-immune thyroiditis have been investigated. In some the iodine uptake was raised to thyrotoxic levels in the early stages and subsequently came down to normal, while in others it was found to be normal or low on the first test though an earlier rise cannot be excluded. High titres of thyroid auto-antibodies were found in most of these cases, but the effect of the auto-immune process on thyroid function has been variable.

Subacute Auto-immune Thyroiditis and Raised ^{131}I Uptake

Case 10.—Girl aged 18. In February, 1957, she felt ill for a few days and rapidly developed a painful and tender thyroid swelling. Malaise, fever, and pain subsided, but the thyroid remained enlarged. In August, 1958, she felt ill again, had a dull ache in arms and legs with paraesthesiae and a feeling of "being bloated"; was irritable, weepy, excessively tired, and unable to work. Past illnesses included pneumonia at 3, mumps at 4, bilious attacks in childhood, and rheumatic fever at 13. There was no family history of goitre.

On examination (Dr. T. M. L. Price, Lewisham Hospital) she was lethargic and pale, apyrexial, with a dry but warm skin. Bizarre subjective symptoms suggested early schizophrenia or hypochondriacal neurosis. There was a diffuse firm thyroid swelling. B.M.R. +13% and +9%; P.R. 80; B.P. 120/70; serum cholesterol 280 mg./100 ml. ^{131}I excretion test: 0–8 hours 16.7%, 8–24 hours 1.1%, 0–48 hours 18.9%. Fraser's 'T' index, 80 (thyrotoxic range); 48-hour P.B.^{131}I, 0.62%/l. Thymol turbidity 4 units, γ-globulins normal. *Thyroid antibodies:*—C.F.T. titre 512, T.R.C. test negative, no precipitins. When patient was referred to the Middlesex Hospital for further study in November, 1958, all peculiar subjective symptoms had subsided and the goitre had decreased in size, the patient remaining euthyroid. The thyroid was still enlarged and firm; estimated weight 35–40 g., with a blowing bruit over the larger right lobe. B.M.R. +9%; cholesterol 220 mg./100 ml.; P.B.I. 10 μg./100 ml. (duplicate 10.2 μg.). ^{131}I excretion had increased to 56%/24 hours with a thyroid uptake of 34% and a 48-hour total plasma content of 0.15%/l., thus showing a substantial decrease in iodine turnover rate three months after onset of the attack. Thymol turbidity 3 units, colloidal gold negative, zinc sulphate 16 units. Serum antibody levels unchanged.

Treatment.—L-Thyroxine 0.2 mg. daily; goitre continued to decrease. She remained well and resumed her job though another slight attack similar to two previous ones occurred in March, 1959. L-E cell test negative; thyroid C.F.T. titre 256.

Case 11.—Woman aged 27. In October, 1955 she had rapid swelling of the thyroid without pain or constitutional symptoms. On examination three weeks after onset there was moderate-sized diffuse firm goitre. B.M.R. −3%; P.R. 68; B.P. 130/70. ^{131}I uptake 70% in three hours, 80% in 24 hours. E.S.R. 35 mm. (Wintrobe); γ-globulins raised to 30.4% = 1.99 g./100 ml.; thymol turbidity 8 units, zinc sulphate 17 units, colloidal gold 5 units. Thyroid swelling subsided in six weeks without treatment. Patient has been under observation for three and a half years, during which time she has remained symptomless with a firm thyroid of normal size. In 1956 ^{131}I uptake was 57%, excretion 31%, 48-hour P.B.^{131}I 0.25%/l. In 1957, B.M.R. −9%; ^{131}I uptake further dropped to 29%, with 48-hour plasma content of 0.1%/l., γ-globulins have been persistently raised; no L-E cells were detected. *Thyroid antibodies:*—C.F.T. titre 32, T.R.C. titre 250. In 1959 thymol turbidity 4 units, zinc 14 units, colloidal gold 2 units. Serum cholesterol 260 mg./100 ml., auto-antibody titre unchanged. So far there are no symptoms or signs suggestive of hypothyroidism.

Case 12.—Woman aged 37. Presented with six weeks' goitre, causing aching neck and back of head. No previous illnesses. Sister had thyrotoxicosis. On examination, clinically euthyroid; smooth diffuse firm goitre, estimated weight 40 g. B.M.R. −7%, P.R. 60, B.P. 110/70. ^{131}I uptake 57% in three hours, 64% in 24 hours, excretion 21%; 48-hour P.B.^{131}I 1.0%/l. *Topography:*—Thyroid shaped and symmetrical. γ-globulins normal, thymol 2 units, zinc 8 units, colloidal gold 1 unit. Thyroid C.F.T. strongly positive, T.R.C. titre 2,500, no precipitins. Treated with thyroid 2 gr. (130 mg.) daily; after one and a half years thyroid still very firm; 25–30 g. Antibodies have almost disappeared and treatment has been stopped in order to reassess thyroid function.

Comment

Three features are common to the cases described in this group. The onset of goitre was rapid and sometimes painful. The iodine turnover rate was increased to thyrotoxic levels in the absence of clinical hyperthyroidism, and serum auto-antibody levels were high soon after onset. In two cases, thyroidectomized seven and five months after onset, histology was that of an active and advanced lymphadenoid process, while in the other three patients the lesion appears to be less destructive and shows a fluctuating or remitting clinical course.

Subacute Auto-immune Thyroiditis with Normal Iodine Uptake

Case 13.—Man aged 69. In December, 1956, he felt ill, and within one to two days had a thyroid swelling with pain in the neck and malaise. Two weeks later jaundice appeared and the patient remained ill and icteric for six weeks. On admission in February, 1957 (Professor D. W. Smithers), the thyroid was greatly enlarged, tender, hard, with a broad isthmus. There was no jaundice, but the liver was enlarged three fingerbreadths below costal margin, firm, and non-tender. *Investigations:*—B.M.R. +2%, ^{131}I uptake 30%/24 hours, plasma clearance rate 31 ml./min., serum cholesterol 320 mg./100 ml., E.S.R. = 136 mm. (1 hour Westergren). Thymol 9 units, zinc 16 units, colloidal gold positive; γ-globulins raised; serum bilirubin 1.2 mg./100 ml.; alkaline phosphatase 25 units. Wassermann and Kahn negative; L-E cell test not done. *Thyroid antibodies:*—Precipitin test positive. Liver biopsy showed active progressive hepatitis, and thyroid biopsy revealed extensive lymphadenoid replacement with altered thyroid epithelium and loss of normal architecture. *Follow-up:*—Goitre gradually disappeared with thyroid medication. In August, 1958, the liver condition appeared quiescent. Thyroid antibody tests were repeated and extended. Precipitin was still positive though weaker than before. T.R.C. tests still positive at 2,500,000 serum dilutions. Thyroid C.F.T. titre 128. Serum also fixed complement with human liver to a titre of 512 and kidney to 128.

Comment

This patient's thyroid-function tests were done 10 weeks after the onset of symptoms, and it may well be that a raised uptake would have been found earlier on. He was euthyroid despite an advanced lymphadenoid lesion; this is often seen in patients who develop a large goitre with continually regenerating epithelium. The associated hepatitis with complement-fixing auto-antibodies against liver and kidney antigens is of particular interest in this case. This association is by no means rare, and several cases have been studied immunologically (Doniach, Roitt, and Hudson, 1959). In one similar case, where myxoedema and progressive hepatitis developed simultaneously and in which the patient's serum contained thyroglobulin precipitins and C.F. antibodies against thyroid gland, liver, and other organs, L-E cells were also present. These patients thus fall into the group described by Mackay and Larkin (1958) under the name of lupoid hepatitis.

It is not clear whether the hepatitis in such cases is of viral origin and if so, whether the virus infection initiates the auto-immune phenomena. In the present case thyroiditis and hepatitis developed fairly acutely and simultaneously, and on clinical grounds the hypothesis of a viral aetiology might have been entertained. However, the hepatitis virus has never been reported to cause thyroiditis, and since it cannot be cultured or identified by a specific test there is no means of proving its possible role in the causation of this type of progressive hepatitis.

Subacute Auto-immune Thyroiditis with Low Iodine Uptake

Case 14.—Woman aged 50. In February, 1957, she felt unwell and noticed an aching swelling in the neck giving rise to a choking sensation. Neck pain continued on and off for three months and was accompanied by progressive onset of myxoedema. In May, 1957, B.M.R. −30% and ^{131}I uptake 2%/24 hours. The thyroid was firm but not enlarged. Precipitins and C.F. antibodies were present in high titre.

Case 15.—Woman aged 32. In October, 1957, she had a febrile illness lasting 10 days, during which the presence of a small soft goitre was noted (patient's sister also has a non-toxic goitre), the patient being euthyroid. Goitre became firmer after a second infection, diagnosed as influenza, in November, 1957, and she began to feel cold and slowed down. By mid-December she was myxoedematous and had a firm diffuse goitre, estimated weight 35 g. B.M.R. −22%; serum cholesterol 540 mg./100 ml.; ^{131}I uptake 3%, excretion 52%/24 hours. Thymol turbidity 6 units, zinc sulphate 19 units, colloidal gold test negative, γ-globulins raised. Precipitins and T.R.C. test strongly positive, C.F.T. negative. On thyroid, 2 gr. (130 mg.) daily, symptoms subsided and goitre decreased to about 20 g. but remained firm. Myxoedema promptly returned when patient failed to take thyroid tablets for one month.

Case 16.—Woman aged 36. Two weeks before admission, general malaise followed by a stiff neck with intermittent

pain in the throat since. One week before admission noticed hard swelling in neck. On examination: clinically almost euthyroid, irregularly enlarged firm thyroid, estimated weight 50–60 g., slightly tender. B.M.R. −15%; serum cholesterol 200 mg./100 ml.; ^{131}I uptake 10%/24 hours. E.S.R. 90 mm. (Westergren); γ-globulins increased; thymol turbidity 6 units; colloidal gold 4 units. Precipitin test strongly positive, C.F.T. positive. In response to cortisone patient became hypothyroid, but when given thyroid hormone all symptoms were relieved and goitre regressed.

Comment

Though the three patients in this group had a fairly rapid onset of symptoms, they already had irreversible thyroid failure when tested, which perhaps explains their low iodine uptake and high precipitin titres. It is possible that auto-immune lesions were present before the onset of the febrile episodes. These febrile illnesses may be interpreted as either toxic reactions connected with the thyroiditis process itself or the result of incidental upper respiratory virus infections. Such infections appear to have a boosting or activating effect in established cases of Hashimoto's disease, an effect which was particularly noticeable in Cases 1 and 9 reported above and Cases E. M. and J. C. described previously (Doniach and Hudson, 1957).

Cases Presenting Diagnostic Problems

Case 17.—Woman aged 51. Attended hospital for menopausal depression associated with financial and family difficulties. No symptoms suggestive of thyroid dysfunction. Routine examination revealed slightly enlarged firm left thyroid lobe, estimated weight 25 g., with finely irregular surface, and a barely palpable right lobe. B.M.R. −7%; ^{131}I uptake 34%; urine excretion 56%/24 hours; 48-hour plasma activity 0.87%; P.B.^{131}I 0.68%/l. Topography showed maximum activity over enlarged left lobe. These findings suggested a single functioning adenoma, but T_3 given for 10 days caused complete suppression of neck uptake. *Thyroid antibodies:*—C.F.T. titre 128, T.R.C. test negative. *Follow-up:*—No symptoms or signs of thyroid deficiency have appeared in two years, but the patient has increased in weight from 8 st. 12 lb. to 9 st. 9 lb. (56.2 to 61.2 kg.) and her serum cholesterol is now 350 mg./100 ml.; γ-globulins and flocculation values normal and antibody levels unchanged. This patient might well develop very gradual loss of thyroid function over the next few years.

Case 18.—Woman aged 47. Increasing nervousness, palpitations, hot flushes, and headaches since hysterectomy two years previously, with appearance of a goitre. On examination very firm diffuse painless thyroid enlargement, estimated weight 45 g. P.R. 120, B.P. 170/105; moist skin and tremor of hands. B.M.R. +17%; ^{131}I uptake 51%, excretion 46%/24 hours; 48-hour plasma activity 0.83%; P.B.^{131}I 0.63%/l. These findings suggested mild hyperthyroidism, and a therapeutic trial of carbimazole 30 mg. daily was given. On this the goitre increased in size and symptoms were aggravated. Diagnosis of lymphadenoid goitre was then considered, and on further investigation it was found that the iodine uptake was depressed to less than 2%/24 hours with T_3. Thymol turbidity 4 units, zinc sulphate 13 units, colloidal gold 1 unit. Thyroid complement-fixation positive, T.R.C. negative. Follow-up for two years on thyroid 2 gr. (130 mg.) daily produced slight improvement though patient still has headaches and nervousness, attributed to hypertension and emotional factors. Thyroid still firm and moderately enlarged, with occasional attacks of aching which radiates to ears and back of head. Antibody tests now negative, thymol 2 units, zinc sulphate 11 units, gold test negative.

Comment

Lymphadenoid goitres are occasionally asymmetrical, and when a rapid iodine turnover is associated with maximum uptake over the swelling as in Case 17, a hot nodule may be simulated, particularly in view of the high 48-hour P.B.^{131}I obtained in both conditions. Hot nodules, however, behave like thyrotoxic tissue in that their iodine uptake cannot be suppressed by T_3 in Werner's test. Moreover, they are usually smooth, whereas lymphadenoid goitre tends to have a finely bosselated surface; antibody tests are usually negative in single functioning adenomas.

Thyrotoxicosis may coexist with Hashimoto's disease, but Case 18 only mimicked hyperthyroidism, as seen from the result of the T_3 suppression test and from the lack of response to antithyroid drugs. The puzzling feature in this patient is the low titre of auto-antibodies and their complete disappearance following hormone treatment while the thyroid gland still retains the features of a typical lymphadenoid goitre. Other antibodies not detected by routine methods may exist.

Case 19.—Woman aged 27. Gradual onset of painless goitre three months after delivery of second child, with increased tiredness. No goitre noticed after first pregnancy or at puberty and no family history of thyroid disease. On examination, moderately firm diffuse goitre, estimated weight 60–80 g., with well-marked pyramidal lobe. No bruit or tenderness. B.M.R. −3%; ^{131}I tests not done in view of breast-feeding; γ-globulins slightly raised, thymol turbidity 6 units; zinc sulphate test 11 units, colloidal gold 1 unit. *Thyroid antibodies:* C.F.T. titre 64, T.R.C. titre 250, no precipitins. Diagnosed as early lymphadenoid goitre and treated with thyroid 2 gr. (130 mg.) daily. Thyroid gland returned to normal size within six months though C.F. antibodies persisted. Treatment continued for 16 months, then stopped for three months to reassess thyroid function: B.M.R. = −2%, serum cholesterol 230 mg./100 ml., thymol turbidity 3 units, zinc sulphate 11 units. ^{131}I uptake 38%/24 hours, excretion 55%; 48-hour plasma activity 0.06%/l. Topography: symmetrical and thyroid-shaped. 10 units of T.S.H. intramuscularly increased the thyroid uptake to 67.4%/24 hours, suggesting normal thyroid reserve. The accompanying Table shows the complement-fixation results obtained at intervals during the period of observation.

Complement-fixation Titres in Case 19 Over a Follow-up Period of Two Years, Showing Gradual Decrease of Antibodies with Disappearance of Goitre Following Treatment with Thyroid Extract

	Serum Dilutions							
	4	8	16	32	64	128	256	512
November, 1957	4	4	4	4	4	0	0	0
April, 1958 ..	4	4	4	1	0	0	0	0
November, 1958	4	4	4	2	0	0	0	0
May, 1959 ..	4	4	3	1	0	0	0	0

Degrees of haemolysis inhibition in C.F.T. are denoted by numbers 4, 3, 2, and 1, equivalent to 100%, 75%, 50%, and 25% inhibition respectively, using 2 M.H.D. of complement. 0 = negative test.

Comment

The diagnosis in this patient would have been more satisfactorily established had it been possible to do ^{131}I studies or had a needle biopsy been carried out. The character of the goitre, the raised thymol turbidity, and the fairly high C.F. titre were in favour of lymphadenoid goitre, and this was further supported by the disappearance of the goitre with thyroid extract. However, the gland behaved normally three months after stopping treatment, and the antibody titre appears to be declining. This could have been a simple

pregnancy goitre with mild focal thyroiditis which was possibly stimulated by the reactive hyperplasia in the gland. On the other hand, this case may be an example of auto-immune thyroiditis with a remitting course.

Cases of Myxoedema Following Mumps

Serological tests were done in three cases of myxoedema which followed definite attacks of mumps. In the first patient, a woman of 35 who contracted mumps from her children, a painful thyroid swelling lasting three weeks developed together with a classical parotitis. Following this illness she felt tired and lethargic, and when seen six months later she was profoundly myxoedematous, with a ^{131}I uptake of 10% and serum cholesterol 350 mg./100 ml. Thyroid auto-antibodies were demonstrated by the T.R.C. test to a titre of 25,000. The second patient, a man of 32, had severe mumps parotitis with orchitis and became hypothyroid in the next 18 months, though no thyroid pain or swelling had been noted at the time of the acute infection. T.R.C. titre was 250.* The third patient was a woman of 55 who also became myxoedematous following mumps parotitis. In this age-group spontaneous myxoedema is fairly common and the association with mumps may be a coincidence, while in the two younger patients a true connexion seems more plausible.

Discussion

Serological surveys have shown that circulating thyroid auto-antibodies of low titre are present in a small proportion of subjects without overt thyroid dysfunction, especially among middle-aged women. The incidence of these antibodies and of corresponding focal lymphocytic thyroid lesions is substantially higher in patients with any type of goitre, particularly in Graves's disease. In the majority of cases such localized lesions are of no clinical significance, since they appear to remain stationary and do not lead to hormonal failure. Where the lesion progresses to lymphadenoid goitre or to the fibrous atrophy of primary myxoedema, the auto-immune process takes a much more active course, and it is unknown at present what underlying factors differentiate this destructive thyroiditis from the mild, purely histopathological lesion found in so many varieties of underlying goitres and in normal thyroid glands.

It was previously postulated that any leakage into the circulation might set up the formation of auto-antibodies to secluded organ-specific proteins to which no immunological tolerance had developed. This mechanism may well account for the localized lymphoid areas so often seen in compressed thyroid tissue surrounding adenomata or in other situations where the epithelium of distended or overactive acini might be more prone to minor breaches of continuity), but does not provide a complete explanation for the progressive lesion of Hashimoto's disease (Hudson, 1959). This may require the participation of another process such as the establishment of a delayed type of hypersensitivity. Intradermal tests with thyroid extracts performed in patients with auto-immune thyroiditis produced reactions possibly of the delayed type in which erythema and induration became maximal at about 24 hours (Buchanan, Anderson, Goudie, and Gray, 1958; see also Witebsky, 1959). There is also good evidence in the experimental auto-immune diseases that this type of response is essential to the development of histological lesions (Waksman, 1959).

Another factor which was thought to be significant in Hashimoto's disease was the ability of the patient to develop high antibody levels in response to the auto-antigenic stimulus (Doniach and Roitt, 1957). However, Case 9 showed a progressive rise in antibody titres from a low initial level during the period of lymphadenoid involvement (Fig. 2), and similar low antibody titres were found in a number of other patients with Hashimoto's disease during the active phase of thyroid enlargement, which suggests that the progression of the lesion is not the result of high antibody levels but rather the cause of their rise.

Recent preliminary experiments conducted with Professor Pulvertaft suggests another factor which might be implicated in the progressive lymphadenoid lesion. It has been found that fresh Hashimoto serum is cytotoxic to thyroid epithelial cells in tissue culture (Pulvertaft, Doniach, Roitt, and Hudson 1959). It remains to be established whether this cytotoxic agent is an antibody and whether its presence will differentiate between actively progressing thyroiditis and the more quiescent type of lesion.

If leakage of intra-acinar or intracellular antigens plays any part in the initiation of the lymphadenoid lesion then virus infections of the gland should provide ideal conditions for the development of auto-immune thyroiditis, not only because of the extensive breakdown of thyroid acini with release of potential auto-antigens into the circulation but also because of a possible adjuvant effect attributed to viruses in the causation of auto-allergic diseases (Waksman, 1959).

de Quervain's giant-cell thyroiditis, with its rapid onset of a painful goitre accompanied by malaise and fever and its limited course followed by complete recovery, has long been thought to be a virus infection. The recent demonstration of mumps virus in an epidemic of this disease in Israel was followed by the report of a similar case in this country (Felix-Davies, 1958). Apart from serological evidence of mumps infection, this patient also had high titres of antibodies to thyroglobulin and to the C.F. cellular antigen. Since this patient had Graves's disease, she may well have had focal thyroiditis before her mumps infection; her auto-antibody titres rose for two months after onset of the acute episode and then regressed slowly. Three years later her serum still contains a substantial titre of antibodies. Her thyrotoxicosis subsided after the mumps thyroiditis and she has not so far progressed to myxoedema (Felix-Davies, personal communication).

None of the 11 cases of mumps thyroiditis observed by Eylan *et al.* (1957) became myxoedematous, and sera taken from them one to two years after the acute attack gave negative results in the thyroid complement-fixation test, though three of them had traces of thyroglobulin antibodies and gave tanned-cell titres of 25, 25, and 2,500 respectively. (N.B.—We thank Dr. W. R. Trotter for allowing us to test these sera with the T.R.C. method.)

The three myxoedema cases mentioned above, which followed definite attacks of mumps, had thyroid

*These two cases will be reported more fully by their physicians in charge.

auto-antibodies, and it remains a plausible assumption that the mumps virus may have initiated or potentiated the auto-immune lesion which led to thyroid destruction.

de Quervain's disease has been studied extensively, and, though myxoedema has been reported in isolated cases (McConahey and Keating, 1951, Woolner, McConahey, and Beahrs, 1957), Crile, who has followed a large number of subacute thyroiditis cases, proved by needle biopsy, firmly states that it never results in myxoedema unless treated by thyroidectomy or heavy *x*-irradiation (Crile, 1948; Crile and Rumsey, 1950). A similar opinion is held by Volpe and Johnston (1957), who have reported on 56 cases seen in Toronto. It is probable that viruses other than mumps may infect the thyroid and that organisms vary in different countries and from case to case. This possibility is strongly suggested by the geographical distribution of de Quervain's disease which is common in Ohio and Minnesota, and possibly in Toronto, but is rare in Boston and extremely uncommon in London. We have observed only four cases at the Middlesex Hospital in the past three years: two women and two men, all of whom recovered without sequelae. In three of them auto-antibodies of low titre were found 8 to 11 weeks after the acute onset of the disease but vanished again on recovery: two of the sera were tested for mumps antibodies with negative results. The sera of 24 subacute thyroiditis cases, received from other hospitals, in which the diagnosis was supported by a low ^{131}I uptake or biopsy appearances typical of giant-cell thyroiditis, were tested for thyroid auto-antibodies, but results were negative or of low titre only.

Another group of patients whose sera were sent to us for antibody tests were diagnosed clinically as having subacute thyroiditis because of the rapid onset of a painful goitre, but progressed to myxoedema and had high precipitin titres (cf. Druez, Hainaut, Parmentier, and Musin, 1958). In these cases, ^{131}I studies were unfortunately not always available, but when performed within a few weeks of onset they showed a normal uptake. It is therefore likely, on reappraisal, that these patients did not have de Quervain's disease but resemble the cases described in this paper having auto-immune thyroiditis with subacute onset. However, the high precipitin titres present in these patients within weeks of the onset of symptoms is not inconsistent with the possibility of a virus infection activating a pre-existing focal thyroiditis into a more virulent and progressive lesion by setting up a state of delayed hypersensitivity which may affect the tissues. A similar effect may account for the frequent exacerbations with increase in goitre size and thyroid pain seen in established Hashimoto cases following upper respiratory infections. A parallel observation has been made in animals with experimental auto-allergic encephalomyelitis, where intercurrent pneumonia aggravated the lesions of the nervous system (Lee and Schneider, 1957).

The differential diagnosis of this type of "subacute" auto-immune thyroiditis from virus thyroiditis may be based on clinical features, the ^{131}I uptake, and serological tests. In virus thyroiditis the constitutional symptoms are often prominent and the thyroid is acutely painful at the height of the disease; the iodine uptake is completely suppressed for several weeks and no thyroid auto-antibodies can be detected, though mumps antibodies may sometimes be found in the initial stages; there is no rise in γ-globulins, but the α-2 globulins may be elevated as a consequence of tissue breakdown. Auto-immune thyroiditis is occasionally accompanied by thyroid pain, but malaise and fever are uncommon. The patients usually complain of pressure and discomfort in the neck rather than of acute pain. The ^{131}I uptake is often raised above normal, and there is a high 48-hour P.B.^{131}I. Auto-antibodies are present in high titre and give rise to elevated γ-globulins. The E.S.R. and P.B.^{127}I cannot be used as differential criteria in the early stages of the two conditions; later the E.S.R. becomes normal in de Quervain's disease while remaining high in lymphadenoid goitre.

To sum up: patients with de Quervain's disease generally recover with normal thyroid function, and there is as yet no concrete evidence that mumps or other virus infections are implicated in the aetiology of lymphadenoid changes. The problem of these relationships may be solved when thyroiditis cases are investigated by combined studies, which should include ^{131}I and serological tests, needle biopsies, and an intensive search for evidence of specific viral infection.

Conclusions and Summary

Classical lymphadenoid goitre is usually associated with high titre of both thyroglobulin and complement-fixing "anti-microsome" antibodies; some patients have only one or the other of these, and in a small number of cases low titres on completely negative results are obtained by the three methods used to date.

Small lymphadenoid goitres may pass unnoticed and can regress spontaneously before giving rise to so-called "primary myxoedema."

Thyrotoxic patients often have low-titre antibodies and a focal thyroiditis, which remains stationary as a rule but may occasionally take on a progressive course. The process may then develop slowly over a period of many years, ending in myxoedema, or may become extensive in a few months, giving rise to a clinical picture of combined Hashimoto's disease and thyrotoxicosis.

Further studies are required to elucidate the part played by infections in the initiation and maintenance of auto-immune thyroiditis.

Serological studies have revealed "subacute" forms of auto-immune thyroiditis which may either become progressive and end in myxoedema or go into remission without destroying the thyroid gland in the first attack.

The differential diagnosis of this form of auto-immune thyroiditis from virus thyroiditis rests mainly on the clinical features soon after onset, on the ^{131}I uptake and auto-antibody titres in the initial stages, and on the histological picture shown in needle biopsies.

This work would have been impossible without the enthusiastic interest and co-operation of many physicians and surgeons who sent us the sera of their patients, and of pathologists who supplied histological sections of cases examined serologically.

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CUMULATIVE TESTING EXPERIENCE WITH CONSECUTIVE LOTS OF ORAL POLIOMYELITIS VACCINE

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Any vaccine proposed for general and continued use must first satisfy certain laboratory criteria of potency and safety of successive lots produced over a period of time. Oral poliomyelitis vaccine is no exception to this rule, though specific production and testing standards for it have not yet been established.

A number of safeguards are obvious prerequisites to the release of a given production lot for clinical trials. For the most part, these were suggested by experience with other vaccines also prepared in monkey-kidney culture. They are particularly critical in the case of oral poliomyelitis vaccine, which cannot contain a preservative that would control undesirable accompanying micro-organisms. The measures taken must be so applied as to rule out, with a well-founded degree of certainty, the presence in the vaccine of any organism, harmful or not, other than poliovirus. They also must serve to ascertain that the avirulence of the vaccine virus remains unchanged during the course of repeated cultivation, and, under ideal conditions, they should permit measurement of the immunogenic potency of the vaccine batch under test.

With oral poliomyelitis vaccine only the first of these three requirements can be carried out in the laboratory under conditions reflecting reality. The other two questions, of vaccine safety and potency for man via the oral route, could perhaps be answered only by use of the chimpanzee in prohibitive and unrealistic numbers. Consequently, for the answer regarding avirulence, rather artificial and sometimes drastic methods of inoculation of certain species of monkeys, such as the rhesus or the cynomolgus, must be resorted to. And since in the case of oral poliomyelitis vaccine antigenic stimulation is a function of *in vivo* viral multiplication, determination of the virus content of the vaccine is a poor but, in the circumstances, necessary and logical substitute.

Though our laboratories have been engaged in the study of a live virus oral poliomyelitis vaccine since the late 1940s, this report will attempt to deal only with our experience since 1957 in the production and testing of serial lots of vaccine, some of which were used in small- and large-scale clinical trials. This experience will be correlated to the immune status of, and the