

THE ASSOCIATION OF AUTOIMMUNE THYROID

DISEASE AND TYPE 1 DIABETES

ROBERT STUART

GRAY

B.Sc., M.B., Ch.B., M.R.C.P. (U.K.)

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Abstract of thesis

Overt autoimmune hyperthyroidism and hypothyroidism were found three times more commonly in insulin-dependent than in non-insulin-dependent diabetics. Similarly, clinically unrecognised primary autoimmune thyroid failure, as evidenced by elevation of the serum thyrotrophin concentration, was twice as common in insulin-dependent as in non-insulin-dependent diabetics. In contrast to the general insulin-dependent diabetic population, insulin-dependent diabetics with overt and clinically unrecognised autoimmune thyroid disease were characteristically female and middle-aged at the onset of diabetes. Ages at onset of diabetes and of thyroid dysfunction were correlated, suggesting the possibility of a common and coincident pathogenesis. Insulin-dependent diabetics with coexisting autoimmune thyroid disease showed a higher prevalence of HLA-B8, cytoplasmic and complement-fixing islet cell antibodies than those without thyroid disease. Within the insulin-dependent diabetic population, retinopathy was not related to the coexistence of autoimmune thyroid disease.

In diabetics with elevated serum thyrotrophin concentrations but serum total thyroxine concentrations within the normal range, hypothyroidism developed at a rate of 5% per annum in patients with thyroid microsomal antibodies.

Thyroid disease was more common in siblings of diabetics with

thyroid disease than in those of diabetics without thyroid disease. Insulin-dependent diabetes was more common in siblings of diabetics with a personal or family history of thyroid disease than in those of diabetics without such a history. In contrast to the younger, male, insulin-dependent patients, diabetics with coexistent autoimmune thyroid disease showed no seasonal variation in incidence. Thus, the pathogenesis of diabetes, when associated with autoimmune thyroid disease, appears to be dependent upon an inherited predisposition and not on environmental factors.

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CONTENTS

	<u>Page No.</u>
<u>Abstract of thesis.</u>	1
Acknowledgements.	3
<u>Part 1</u>	
Definitions and aims of thesis.	
Chapter 1. Introductions and definitions.	
(1) Classification of diabetes mellitus and autoimmune thyroid disease.	7
(2) Common and contrasting features of type 1 diabetes and autoimmune thyroid disease.	11
(3) Conclusions and aims of thesis.	21
<u>Part 2</u>	
Distribution of overt and clinically unrecognised autoimmune thyroid disease in the diabetic population.	

Chapter 2. Distribution of overt autoimmune thyroid disease in the diabetic population.	23
Chapter 3. Comparison of the prevalence of thyroid antibodies and unrecognised primary thyroid failure in insulin-dependent and non-insulin-dependent diabetics.	29
Chapter 4. Distribution of unrecognised primary thyroid failure in insulin-dependent diabetics.	39
Chapter 5. Natural history of thyroid function in diabetics with impaired thyroid reserve, including an examination of the lipid profiles of diabetics with primary thyroid failure and their response to thyroxine treatment.	47

Part 3

Characteristics of diabetics having coexisting autoimmune thyroid disease.

Chapter 6. Clinical features of diabetics with coexisting autoimmune thyroid disease.	65
Chapter 7. Comparison of retinopathy in insulin-dependent diabetics with and without coexisting autoimmune thyroid disease.	76
Chapter 8. HLA antigen and serological determinants of diabetics with coexisting autoimmune thyroid	80

disease.

Part 4

Aetiology of diabetes in diabetics having coexisting
autoimmune thyroid disease.

Chapter 9. Seasonal incidence of insulin-dependent 94
diabetes in diabetics with and without
autoimmune thyroid disease.

Chapter 10. Family studies in diabetics with and without 107
coexisting autoimmune thyroid disease.

Part 5

Summary 131

REFERENCES

List of published papers

PART 1

CHAPTER 1

(1) Classification of diabetes mellitus.

Before the discovery of insulin, diabetics selected themselves into two groups - those who acquired symptoms acutely, rapidly lost weight, developed ketoacidosis and died, and those who developed symptoms insiduously, were commonly overweight and usually survived many years having severely restricted their carbohydrate intake. Age at onset was evidently a further distinguishing feature since the former group was usually younger than the latter. The discovery of insulin led to the generally accepted clinical distinction between insulin-dependent diabetics and non-insulin-dependent diabetics which, unfortunately, ceded to the more popular differentiation of diabetics into juvenile - and maturity - onset. This was a classification with neither reference to diabetic treatment nor aetiology.

In the early 1970's, all diabetics were considered to suffer from the same disease of homogeneous aetiology but of variable severity and age at presentation. Preliminary evidence in support of an aetiological distinction between insulin-dependent diabetes and non-insulin-dependent diabetes derived from the examination of families of insulin-dependent and non-insulin-dependent diabetics. McDonald (1974) reported equal prevalence of diabetes in grandparents of juvenile-onset, insulin-dependent diabetics and in grandparents of non-diabetic, age-matched controls and subsequent family studies (Irvine et al, 1977c; Cudworth, 1978) suggested that insulin-dependent and non-insulin-dependent dia-

betes "breed true". Confirmation that predispositions to insulin-dependent and non-insulin-dependent diabetes are inherited separately was provided by comparisons of concordance rates for each type of diabetes in monozygotic and dizygotic twins (Barnett et al, 1981).

Comparison of the distribution of HLA antigen frequencies in insulin-dependent and non-insulin-dependent diabetics (Nerup et al, 1974; Cudworth and Woodrow, 1976) showed several significant differences, with family studies (Walker and Cudworth, 1980) confirming association of specific HLA antigens with insulin-dependent but not with non-insulin-dependent diabetes. Since the immune response genes may exist within the HLA system these findings provided support for the view that the aetiology of insulin-dependent diabetes, but not non-insulin-dependent diabetes, might have an immunological basis. Evidence in favour of this concept also derived from the demonstration of humoral (Bottazzo et al, 1974; MacCuish et al, 1974a) and cellular (Nerup et al, 1971; 1973; MacCuish et al, 1974b) sensitivity to pancreatic antigens in insulin-dependent diabetics. Lastly, epidemiological studies suggested that the onset of insulin-dependent diabetes, but not non-insulin-dependent diabetes, might be related to environmental influences (Adams, 1926; Gamble and Taylor, 1969).

With the acknowledgement of an aetiological distinction between non-insulin-dependent and insulin-dependent diabetes, it became apparent that a small proportion of diabetics treated without insulin exhibited HLA, immunological, familial and metabolic characteristics typical of insulin-dependent diabetics. Many of these patients ultimately became insulin-dependent (Irvine et al, 1977a) and it was there-

fore suggested (National Diabetes Data Group, 1979) that those diabetics having the characteristics of insulin-dependent diabetes be described as type 1 diabetics, whilst those having the characteristics of non-insulin-dependent diabetics be described as type 2 diabetics, irrespective of current dependency on insulin.

Classification of auto-immune thyroid disease.

Hashimoto's Thyroiditis

This condition is characteristically found in middle-aged female patients who present, clinically, with a firm, painless, symmetrical goitre often accompanied by a variable degree of primary thyroid failure. The histology of the gland is dominated by diffuse lymphocytic infiltration, commonly leading to the formation of lymphoid follicles (Hashimoto, 1912). The cellular infiltrate includes plasma cells, lymphocytes and often Askanazy cells (Lindsay et al, 1952; Woolner et al, 1959). Fibrosis is frequently present to a varying degree. The parenchyma is often lobulated with a reduction in the number of follicles which may be small, containing little colloid, but may also show hyperplasia. Immunofluorescence studies (Calderon et al, 1973) have shown the presence of antibodies attached to the basement membrane of the follicular cells and these may include thyroid-growth immunoglobulins (Drexhage et al, 1980). A variety of circulating antibodies may be demonstrated including antibodies to thyroglobulin, thyroid colloid and microsomes, and thyroid-growth immunoglobulins.

Primary atrophic hypothyroidism

This is again a condition most commonly found in middle-aged women

presenting with primary thyroid failure unaccompanied by a goitre. Over and above a modest lymphocytic thyroid infiltration and circulating thyroid antibodies usually associated with Hashimoto's thyroiditis, this condition is characterised by fibrous atrophy of the thyroid gland, probably due to the production of antibodies (Drexhage et al, 1981) capable of blocking the action of thyroid- growth immunoglobulins, thyroid-stimulating immunoglobulins and thyrotrophin (TSH).

Graves' Disease

The clinical features of this condition are the result of hypersecretion of thyroid hormones commonly occurring, again, in middle-aged women, many of whom have a goitre. It is associated with the production of human-specific thyroid-stimulating immunoglobulin (Orgiazzi et al, 1976) which cross-reacts with the TSH receptor on the surface membrane of the thyroid cell and which stimulates hyperthyroidism. The goitre may develop as a result of concomitant production of thyroid- growth immunoglobulins (Drexhage et al, 1980) which probably recognise separate antigenic sites on the thyroid cell surface. The histology of the gland reveals hyperplastic follicles with scalloping of the colloid material contained within, and an inconsistent, low-grade lymphocytic infiltration.

Each of these three conditions may be accompanied by ophthalmic pathologies, pre-tibial myxoedema and thyroid acropachy, each of which may have an immunological aetiology. Moreover, an individual patient may develop each of these conditions in sequence. Thus, patients have been described (Irvine et al, 1979) as suffering from primary autoimmune thyroid failure followed by Graves' disease, while a substantial

proportion of patients treated medically for Graves' disease subsequently develop thyroid failure with or without a goitre (Irvine et al, 1977b). Lastly, approximately 25% of Hashimoto goitres disappear completely with thyroid atrophy and fibrosis after prolonged replacement with thyroxine treatment.

It is possible that a proportion of simple, non-toxic goitres are also immunologically derived and are the consequence of the action of thyroid- growth immunoglobulins on normal thyroid tissue. Furthermore, a proportion of multinodular goitres are said to show evidence of thyroid specific humoral and cellular autoimmunity (Mota et al, 1980) although these findings have yet to be confirmed. Nevertheless, the term autoimmune thyroid disease will hereafter be restricted to the three conditions described above - Hashimoto's thyroiditis, primary atrophic hypothyroidism and Graves' disease.

(2) Common and contrasting features of type 1 diabetes and auto-immune thyroid disease.

Histology

Inflammation of islet tissue taken from young diabetics dying shortly after the development of ketotic diabetes was first described as "insulitis" by Von Meyenburg in 1940. Affected islets are usually atrophic and infiltrated to a variable degree with large mono-nuclear cells, sometimes polymorphs and very rarely eosinophils. Plasma cells are conspicuous by their absence. The islet capsule is commonly surrounded by a halo of lymphocytes, extending within the periphery of the islet and cuffing capillaries in the stroma (Warren et al, 1966). Occa-

sionally, the lymphocytic infiltration of the islet parenchyma is particularly florid (Gepts, 1965). Within a year of the onset of diabetes, the inflammatory infiltrate of the islet is no longer seen and the beta cells are atrophied to extinction.

Whereas the histological features of insulitis are fairly uniform, depending largely on the stage of development of the lesion, the histological appearances of thyroid tissue vary according to the nature of the underlying autoimmune thyroid disease. The appearances of the thyroid gland in patients with atrophic hypothyroidism are similar to those of the latter stages of insulitis and one might envisage a greater similarity if thyroid tissue were available in the early stages of the natural history of this condition. In contrast, the histological features of Hashimoto's thyroiditis and Graves' disease may be distinguished from those of insulitis by the florid lymphocytic and plasma cell infiltrates on the one hand, and by parenchymal hypertrophy on the other hand.

Humoral Immunity

Antibodies reactive with a cytoplasmic antigen common to all pancreatic islet cells (Bottazzo et al, 1974; MacCuish et al, 1974a) are present in low titre in most type 1 diabetics at/or pre-diagnosis (Irvine et al, 1976; 1977e). The titre of antibody usually falls within months of the diagnosis of diabetes (Irvine et al, 1977e). Complement fixing antibodies specific for pancreatic beta cells have also been described (Bottazzo et al., 1980). Similarly, patients with autoimmune thyroid disease commonly have circulating antibodies to thyroid microsomes, colloid and thyroglobulin. Such thyroid and islet cell

antibodies may not themselves be cytotoxic and may therefore be of little functional significance. On the other hand islet cell surface antibodies have been described (Lernmark et al., 1978; Freedman et al., 1979) which preferentially bind to the beta cell and are found to correlate with islet cell cytotoxic antibodies which are complement-binding (Dobersen et al., 1980). Complement-binding beta cell surface antibodies may impair the release of insulin from those cells in response to glucose (Kanatsuna et al., 1981; Sai et al., 1981). Antibodies capable of influencing pancreatic islet cell function have yet to be described (other than those causing direct cell damage) in diabetics, whereas antibodies capable of stimulating the thyroid, blocking the TSH receptor and promoting thyroid growth have all been documented in patients with autoimmune thyroid disease (Orgiazzi et al., 1976; Zacharija et al., 1980; Drexhage et al., 1980).

Lastly, immune complexes have been described in the serum of patients with autoimmune thyroid disease (Calder et al., 1974) and in type 1 diabetics (Di Mario et al., 1980). It has been suggested (Calder et al., 1975; Irvine et al., 1978a) that K cells, armed with immune complexes, may be responsible for the tissue damage sustained by each cell type (i.e. insulinitis and thyroiditis), resulting in type 1 diabetes and hypothyroidism, respectively.

Cell-Mediated Immunity

The proportion of circulating T and B lymphocytes in type 1 diabetics is normal (MacCuish et al., 1974c), suppressor T cell activity is reduced (Pozzilli et al., 1983) while K cell activity is increased (Pozzilli et al., 1979). Leucocytes from type 1 diabetics exhibit

hypersensitivity to pancreatic antigen as demonstrated by migration inhibition studies (Nerup et al., 1971; 1973; MacCuish et al., 1974b). Intra-cutaneous injection of a homogenate of duct-ligated porcine pancreas has been shown (Nerup et al 1971) to induce a delayed-type hypersensitivity reaction in a small group of type 1 diabetics who showed leucocyte migration inhibition in response to the same antigen. Lymphocytes from newly presenting type 1 diabetics show significant transformation when challenged with bovine and porcine insulin in apparently half the patients thus tested (MacCuish et al., 1975).

Parallel studies in patients with autoimmune thyroid disease have yielded similar findings. Thus, the proportion of T and B lymphocytes is similar to that found in controls (Urbaniak et al., 1974; Calder et al., 1976), while suppressor T cell function is impaired (Okita et al., 1981) and K cell activity increased (Calder et al., 1976). Leucocyte migration inhibition tests are positive in response to thyroid antigens in patients with Hashimoto's thyroiditis and Graves' disease (Soberg and Hallberg, 1968; Calder et al., 1972; Lamki et al 1973). Greater migration inhibition is found in Hashimoto patients whose goitre fails to respond to thyroxine treatment (Wartenberg et al., 1973). Intracutaneous injection of thyroid extract in patients with Hashimoto's thyroiditis will induce a delayed hypersensitivity reaction (Buchanan et al., 1958; Soberg and Hallberg, 1968). Lymphocytes from Hashimoto's, but not Graves' disease patients have been shown to undergo blast transformation on exposure to thyroglobulin (Lycette and Pearman, 1965; Ehrenfeld et al., 1971; Delespesse et al., 1972). Lymphocytes from patients with Hashimoto's disease are cytotoxic to thyroid cell monolayers (Laryea et al., 1973), chicken red blood cells coated with thyroglobulin

(Calder et al., 1973) and heterologous cells coated with thyroglobulin and thyroid microsomes (Podleski, 1972).

It should be noted that most studies of cell-mediated immunity in autoimmune thyroid disease have concentrated on patients with Hashimoto's thyroiditis. Inconsistent findings in relation to Graves' disease may reflect varying degrees of disease activity. Patients with atrophic hypothyroidism have not received the same attention although within the spectrum of autoimmune thyroid disease, they may represent the closest pathological analogy to the insulinitis of type 1 diabetes.

HLA Analyses

Population studies (Cudworth et al., 1982) have shown an increased incidence of two distinct HLA axes in the insulin-dependent diabetic population, DW4, DR4, A2, BW62, CW3 and DW3, DR3, A1, B8, CW7. The former axis tends to be associated with an earlier age at onset, high insulin antibody titres and possibly an increased risk of microangiopathy (Dornan et al., 1982). The latter tends to be associated with low insulin antibody titres and relative protection from development of microangiopathy. Family studies confirm segregation of HLA type and predisposition to insulin-dependent diabetes within affected families, as might be anticipated (Cudworth et al., 1982).

The HLA axis DW3, DR3, A1, B8 is over represented in patients with Graves' disease (McMichael et al., 1975; Thorsby et al., 1975) and primary atrophic myxoedema (Irvine et al., 1978b; Moens et al., 1979) whereas DR5 has been shown to be associated with Hashimoto's thyroiditis (Weissel et al., 1980). Further heterogeneity has been demonstrated

within the population of patients with Graves' disease. Thus, those whose condition tends to relapse following medical treatment are more commonly DR3, A1, B8 than those remaining in remission (Irvine et al., 1977d; Bech et al., 1977). Similarly, coincident ophthalmopathy is associated with DR3, B8 in patients with Graves' disease (Schleusener et al., 1983).

Familial Predisposition

Acceptance of the aetiological distinction between type 1 and type 2 diabetes has simplified the interpretation of studies of inheritance of diabetes. Thus, twin studies show (Barnett et al., 1981) that approximately half of monozygotic twins are concordant for insulin-dependent diabetes, whereas non-identical twins exhibit a much lower concordance rate of approximately 10%, confirming the strength of the genetic predisposition to type 1 diabetes. Examination of the prevalence of insulin-dependent diabetes in first degree relatives of insulin-dependent diabetics (Walker et al., 1980; Wagener et al., 1982; Kobberling et al., 1980) shows the risk to be approximately similar in siblings (3.3 - 5.7%), parents (2.6%) and children (1.5%).

Twin studies have yielded parallel confirmation of the inherited predisposition to Graves' disease (Harvald and Hauge, 1956; Hassan et al., 1956), Hashimoto's thyroiditis (Irvine et al., 1961; Zaino and Gerra, 1964) and primary atrophic hypothyroidism (Hennen and Dodinval, 1965). The concordance rate for Graves' disease is of the same order as that found for insulin-dependent diabetes (Harvald and Hauge, 1956; Hassan et al., 1956; Vogel, 1959), while figures for Hashimoto's disease and primary atrophic hypothyroidism are not yet available.

Family studies also indicate an increased risk of autoimmune thyroid disease in close relatives of patients with Graves' disease (Bartels, 1941; Martin and Fisher, 1945; Howel-Evans et al., 1967), primary atrophic hypothyroidism (Hall, 1967) and Hashimoto's thyroiditis (Hall and Stanbury, 1967; Chopra et al., 1977). Indeed, the risk of thyroid disease in close relatives of probands with autoimmune thyroid disease appears to be approximately twice that for insulin-dependent diabetes in relatives of insulin-dependent diabetics.

A further important difference between the patterns of inheritance of insulin-dependent diabetes and autoimmune thyroid disease is that the sexes are approximately equally represented in affected relatives of insulin-dependent diabetics who themselves show roughly equal sex incidence. In contrast, affected relatives of subjects with autoimmune thyroid disease exhibit marked female preponderance, as do the probands, irrespective of type of autoimmune thyroid disease.

The prevalences of islet cell and thyroid antibodies are increased in close relatives of insulin-dependent diabetics (Irvine et al., 1977e; Gorsuch et al., 1981; Ginsberg-Fellner et al., 1982) and patients with autoimmune thyroid disease (Howell-Evans et al., 1967; Chopra et al., 1977; Hall et al., 1960; Mather et al., 1980), respectively. Once again, islet cell antibody positive relatives show a sex ratio of approximately 1:1, whereas thyroid antibody positive relatives show female preponderance.

The modes of inheritance of insulin-dependent diabetes and of autoimmune thyroid disease remain undefined. It is thought likely that a polygenic mode of inheritance rather than a simple autosomal dominant

or recessive mode of inheritance may be operative.

Aetiology and the Environment

Seasonal variation in the incidence of juvenile-onset type 1 diabetes was first reported by Adams (1926) and subsequently confirmed by Gamble and Taylor (1969). These observations, together with the relationship between peaks in age incidence of type 1 diabetes and exposure to new school environments (Gamble et al., 1973) led to the hypothesis of a viral aetiology for type 1 diabetes. Further support for this hypothesis has derived from the temporal clustering of onset of type 1 diabetes in twins (Nelson et al., 1975) and sibships (Gamble, 1980). Direct evidence for a viral aetiology is provided by reports of type 1 diabetes following specific viral infections (Burgess et al., 1974; Forrest et al., 1971; Yoon et al., 1979) and by an increased prevalence of Coxsackie B4 antibodies in established type 1 diabetics (Gamble et al., 1969). Certain viruses may be demonstrated (Craighead and McLane, 1968) to be beta cytotropic and to cause insulinitis analogous to that seen in human type 1 diabetics. Of further interest, is the observation that the susceptibility to insulinitis depends on genetic factors as well as prior exposure to streptozotocin (Toniolo et al., 1980) and testosterone (Helgason and Jonasson, 1981; Helgason et al., 1982). Other environmental agents implicated in the pathogenesis of type 1 diabetes include rodenticides (Karam et al., 1980) and N-nitroso compounds (Helgason and Jonasson, 1981; Helgason et al., 1982).

By contrast, the evidence for an environmental pathogenesis of autoimmune thyroid disease is lacking (Hadden and McDevitt, 1974). An unconfirmed report (Volpe, 1979) suggested there to be an increased

frequency of antibodies to influenza B and mumps viruses in thyrotoxic patients. Whereas systemic viral infections are not uncommonly accompanied by self-limiting subacute thyroiditis, established autoimmune thyroid disease very rarely follows such an illness (Joasoo et al, 1975; Werner, 1979). Isolation of viral particles from diseased thyroid tissue has not been convincingly demonstrated despite the wealth of available material obtained surgically. A single report (Farid et al., 1980) of a seasonal incidence of HLA-B8 positive Graves' disease remains unconfirmed. Other environmental agents are likely to account for only the small minority of cases of thyroid disease. While "stress" is frequently implicated in the pathogenesis of Graves' disease, direct evidence in favour of such a pathogenesis is lacking (Hadden and McDevitt, 1974). In the face of cumulative evidence against an environmental cause of autoimmune thyroid disease, the fact remains that only approximately half the monozygotic co-twins of patients with Graves' disease or thyroiditis develop the same condition. Moreover, there are reports of one twin developing Graves' disease while the other develops thyroiditis (Jayson et al., 1967; Kidd et al., 1980). Thus, non-genetic factors must influence the probability of developing these conditions although the responsible agent(s) remain undetermined.

Animal Models

A number of naturally occurring animal models of type 1 diabetes have been described. The BB/W rat emerged as a spontaneous mutation within a breeding colony. Approximately half of such animals develop insulin-dependent diabetes at 60-180 days of age and may be found to have a round cell pancreatic islet infiltrate (Nakhooda et al., 1976).

They are immunologically incompetent (Naji et al., 1981; Bellgrau et al., 1982) while neonatal thymectomy or immunosuppression with anti-lymphocytic serum prevents the development of insulinitis/diabetes (Like et al., 1979; 1981). BB/W rats raised in a sterile gnotobiotic environment develop diabetes with a frequency similar to that observed in an unprotected environment (Rossini et al., 1979). Although this does not exclude the possibility of a vertically transmitted infective agent, e.g. virus, being responsible for the pathogenesis of insulinitis, it is possible that this animal model represents a pure autoimmune disease, being independent of environmental influences. A similar pathogenesis has been offered as cause of the insulinitis found in the NZB mouse which develops other autoimmune phenomena such as lupus and nephritis (Melez et al., 1980; Kolb et al., 1980).

Insulinitis may also be induced in animals by immunisation of heterologous or homologous insulin (Le Compte et al., 1966; Grodsky et al., 1966) with Freund's adjuvant or injection of endocrine pancreas with Freund's adjuvant. Passive immunisation by injection of heterologous anti-insulin serum will also induce insulinitis (Lacy et al., 1965).

Spontaneous development of thyroiditis occurs in the Obese strain of white Leghorn (OS) chickens whose thyroid histology closely resembles that of Hashimoto's thyroiditis. Such animals have circulating thyroglobulin antibodies whose titre correlates with severity of disease (Wick et al., 1974). Local production of thyroglobulin antibody by plasma cells infiltrating the thyroid gland has been demonstrated (Schauenstein and Wick, 1974). In contrast to the BB/W rat, neonatal thymectomy enhances the degree of thyroiditis whereas bursectomy reduces

the severity of the thyroiditis. Spontaneous autoimmune thyroiditis, whose histology is similar to that of Hashimoto's thyroiditis, is also reported in closed colonies of Beagle dogs, (Fritz et al., 1970) thymectomised and whole-body X-irradiated Wistar rats (Penhale et al., 1973) and Buffalo strain rats (Glover and Reuber, 1968). Lastly, the BB/W rat is also recognised to develop thyroiditis spontaneously, and concomitantly with insulinitis (Sternthal et al., 1981).

Immunisation with thyroid extract will induce experimental thyroiditis having characteristic thyroid histology, circulating thyroglobulin antibodies and delayed-type skin reactions to thyroglobulin (Wick, 1975). In contrast to the cellular infiltrate of Hashimoto's thyroiditis and the spontaneous thyroiditis of OS chickens, plasma cells and germinal centres are rarely seen, while microsomal antibodies are only found in the monkey animal model (Andrada et al., 1968). An experimental model of Graves' disease has yet to be described.

(3) Conclusions and aims of thesis.

Type 1 diabetes and autoimmune thyroid disease thus share very many characteristics. They have similar histologies, humoral and cellular immune characteristics, HLA antigen distributions, and familial predisposition, while animal models exhibit parallel features and even coexisting insulinitis and thyroiditis. Nevertheless, several striking differences are evident between these two conditions. Thus, whereas the evidence in favour of an environmental precipitant to the pathogenesis of type 1 diabetes is irrefutable, no such reliable evidence yet exists in respect of autoimmune thyroid disease. Alternatively, the familial risk of autoimmune thyroid disease appears to be considerably stronger

than that of type 1 diabetes, and whereas female preponderance is characteristic of subjects with autoimmune thyroid disease, the sexes are approximately equally represented when considering type 1 diabetics. Type 1 diabetes is predominately a disease of childhood or adolescence whereas autoimmune thyroid disease affects middle-aged subjects. Within the category of autoimmune thyroid disease, a variety of differing pathologies of distinct clinical, histological, HLA and immunological expression is evident. At first sight, this may be seen as a further major distinguishing feature between type 1 diabetes and autoimmune thyroid disease since a greater degree of homogeneity appears to be evident within the type 1 diabetic population.

The aims of the studies embodied in this thesis were

(a) to establish the frequency with which overt and clinically unrecognised autoimmune thyroid disease are found in the diabetic population.

(b) to investigate the clinical, metabolic, immunological, familial and HLA antigen characteristics of diabetics with coexistent autoimmune thyroid disease and to compare these characteristics with those of diabetics without thyroid disease.

(c) to consider possible aetiological factors responsible for the pathogenesis of diabetes when associated with autoimmune thyroid disease.

PART 2

CHAPTER 2

The distribution of overt autoimmune thyroid disease in the diabetic population.

In view of the obvious similarities between the pathological processes culminating in autoimmune thyroid disease and type I diabetes, the distribution of overt autoimmune thyroid disease in the diabetic population was investigated. The main purpose of this enquiry was to compare the prevalence of clinically evident autoimmune thyroid disease in insulin-dependent (type 1) diabetics with that in non-insulin-dependent (type 2) diabetics.

Subjects and Methods

The distribution of thyroid disease was established by

- (a) interviewing patients and
- (b) examining case records of 963 diabetics consecutively attending the Diabetic and Dietetic Department, Royal Infirmary, Edinburgh. Four hundred and thirty five patients (196 males, 239 females) were treated with insulin, 330 (152 males, 178 females) were treated with oral hypoglycaemic agents (OHA) and 198 (111 males, 87 females) were treated with diet alone.

Where a history of thyroid disease was reported by the patient, his/her hospital and general practice records were scrutinised. A history of thyroid disease was only accepted in those patients in whom

biochemical proof of hyper- or hypothyroidism had been obtained. Hyperthyroidism was attributed to autoimmune Graves' disease on the basis of clinical, histological and/or radioisotope scanning evidence whereby patients with an overactive nodular goitre were excluded. Hypothyroidism was always considered to be due to autoimmune thyroiditis in the absence of contradictory evidence.

The data were submitted to χ^2 analysis.

Results

Table 1 shows the distribution of autoimmune thyroid disease in the diabetics studied, according to age, sex and insulin-dependency. Autoimmune thyroid disease was found in 32 (7.4%) insulin-dependent diabetics and 12 (2.3%) non-insulin-dependent diabetics ($\chi^2 = 9.6$, $p < 0.02$). The prevalence of autoimmune thyroid disease was greater in female (10.9%) than in male (3.1%) insulin-dependent diabetics ($\chi^2 = 40.3$, $p < 0.01$), and greater also in female (3.8%) than male (1.5%) non-insulin-dependent diabetics ($\chi^2 = 30.1$, $p < 0.01$). Table 2 shows the prevalence of hyper- and hypothyroidism in the diabetics according to diabetic treatment and sex, and includes comparative data obtained by Tunbridge et al (1977a) in a non-diabetic control population ($n = 2779$). Of the 963 diabetics interviewed 30(3.1%) had Graves' disease and 14(1.4%) had autoimmune primary thyroid failure.

Discussion

A clinical association between thyroid disease and diabetes has been suspected for many years. However the evidence in favour of such an association is based upon a small number of anecdotal reports of

concurrent diabetes and thyroid disease occurring in the same individual or his family (Hayles et al., 1959; Perlman, 1961; Solomon et al., 1965; Crome et al., 1967). Where the prevalence of hypothyroidism in the diabetic population has been studied (Hecht and Gershberg, 1968; Ganz and Kozak, 1974), no attempt has been made to distinguish between insulin-dependent and non-insulin-dependent diabetics. As a result, the strikingly high incidence of hypothyroidism in the insulin-dependent diabetic population was obscured. Nabarro et al (1979) reported the incidences of hyper- and hypothyroidism in insulin deficient diabetics to be 3.0 and 2.4% respectively, and these figures are very similar to our own of 5.3 and 2.1%. Comparative data from a non-diabetic control population are provided by a study (Tunbridge et al., 1977a) of the prevalence of overt thyroid disease in a community from the North-East of England of whom 0.9 and 0.5% had hyper- and hypothyroidism. Thus, it may be seen that clinically evident hyper- and hypothyroidism are approximately four times as common in the insulin-dependent diabetic population as in non-diabetic controls.

In contrast, the distribution of overt autoimmune thyroid disease is not appreciably greater in the non-insulin-dependent diabetic population than in controls given the fact that the non-insulin-dependent diabetics in the present study were considerably older than the majority of the controls taken from the Whickham survey. Thus, a clinical association has been demonstrated between autoimmune thyroid disease and insulin-dependent diabetes, but not non-insulin-dependent diabetes, which complements those other features common to both insulin-dependent diabetes and autoimmune thyroid disease as described in Chapter 1.

Within the population of insulin-dependent diabetics, the risk of developing co-existing autoimmune thyroid disease might be randomly distributed. However, it appears that middle-aged, female, insulin-dependent diabetics exhibit a particularly high incidence of concomitant thyroid disease. The significance of this selective association will be discussed in subsequent chapters of this thesis.

TABLE 1

Prevalence of overt autoimmune thyroid disease in diabetics
according to age, sex and insulin-dependency

	Age (yr)						Total
	<30	30-39	40-49	50-59	60-69	>69	
<u>Insulin-dependent diabetics</u>							
Male	0/66	0/34	0/23	3/40	2/21	1/12	6/196
	0%	0%	0%	7.5%	9.5%	8.3%	3.1%
Female	0/61	5/31	3/32	5/38	8/39	5/38	26/239
	0%	16.1%	9.4%	13.2%	20.5%	13.2%	10.9%
<u>Non-insulin-dependent diabetics</u>							
Male	0/5	0/11	0/32	1/69	1/85	0/61	2/263
	0%	0%	0%	1.5%	1.2%	0%	0.8%
Female	0/7	0/3	1/18	1/51	5/98	3/88	10/265
	0%	0%	5.6%	2%	5.1%	3.4%	3.8%

TABLE 2

Comparison of prevalence (%) of overt autoimmune hyperthyroidism and hypothyroidism in diabetics and controls

	<u>Hyperthyroid</u>	<u>Hypothyroid</u>
<u>Insulin-dependent diabetics</u>		
Male	2.6	0.5
Female	7.5	3.4
<u>Non-insulin-dependent diabetics</u>		
Male	0.8	0
Female	1.9	1.9
<u>Controls*</u>		
Male	0.2	<0.1
Female	1.5	1.0

* data derived from Tunbridge et al (1977a)

CHAPTER 3

A comparison of the prevalences of thyroid antibodies and unrecognised primary thyroid failure in insulin-dependent and non-insulin-dependent diabetics.

The prevalence of thyroid cytoplasmic antibodies is greater in insulin-dependent diabetics than controls (Landing et al., 1963; Irvine et al., 1970; Whittingham et al., 1971; Neufeld et al., 1980). The risk is greatest for females and the difference in prevalence between diabetics and controls is most marked in younger patients (Irvine et al., 1970; Whittingham et al., 1971). In both insulin-dependent diabetics and controls, the prevalence of thyroid cytoplasmic antibodies increases with age. Duration of diabetes and age at onset of diabetes appear to have no independent influence on the risk of having thyroid cytoplasmic antibodies (Irvine et al., 1970). However, one report (Whittingham et al., 1971) suggests a decline in prevalence of thyroid cytoplasmic antibodies in elderly, insulin-dependent diabetics of long duration. The same investigators observed vascular disease to be particularly common in thyroid antibody positive young diabetics with long-term insulin dependency and proposed that the decline in prevalence of thyroid cytoplasmic antibodies in long-standing, elderly insulin-dependent diabetics was due to selective premature mortality of thyroid antibody positive insulin-dependent diabetics in middle-age.

The distribution of thyroglobulin antibodies parallels that of thyroid cytoplasmic antibodies with insulin-dependent diabetics exhibiting a higher prevalence than controls, both groups showing increasing prevalence with age and female insulin-dependent diabetics exhibiting

particular susceptibility (Irvine et al., 1970; Simkins, 1968). In contrast to the increased frequency with which thyroid cytoplasmic and thyroglobulin antibodies are found in insulin-dependent diabetics, neither antibody is associated with non-insulin-dependent diabetes (Irvine et al., 1970; Whittingham et al., 1971). This observation lent early support to the view (now widely held) that insulin-dependent and non-insulin-dependent diabetes might have differing pathogeneses. The appearance of thyroid antibodies was clearly not the result of disordered carbohydrate metabolism, per se.

The heterogeneous distribution of thyroid antibodies within the insulin-dependent diabetic population is intriguing and suggests varying susceptibility to coincident thyroid autoimmunity based on age and sex. However, interpretation of cross-sectional prevalence data is handicapped not only by the distortion introduced by putative selective factors as proposed by Whittingham et al (1971), but also by the uncertainty of the relevance of the appearance of thyroid antibodies. Thus, while it is recognised (Goudie et al., 1959; Bastenie et al., 1967) that in the control population thyroid antibodies are associated with thyroiditis in the absence of clinical thyroid disease, they may appear transiently and be of little functional or pathological significance (Hawkins et al., 1980; Tunbridge et al., 1981).

In view of the uncertainty of the functional significance of thyroid antibodies and therefore of the relevance of their distribution within the diabetic population, a study of thyroid function was undertaken in a large group of unselected diabetics who had no history of thyroid disease. The same patients were examined for thyroid antibodies

and the relationship between antibodies and thyroid function was critically evaluated. Particular attention was paid to the comparative prevalence of unrecognised thyroid failure in the insulin-dependent and non-insulin-dependent diabetic populations.

Subjects and Methods

Three hundred and forty-seven diabetics were studied including 184 insulin-dependent diabetics (91 males, 93 females), 101 patients (44 males, 57 females) treated with OHA and 62 patients (34 males, 28 females) treated by diet alone. The mean \pm SE ages of the three groups of subjects were 49.0 ± 1.0 , 53.0 ± 0.9 and 52.0 ± 0.9 years with durations of diabetes of 17.4 ± 0.6 , 7.3 ± 0.6 and 4.1 ± 0.6 years, respectively. Of the patients treated with OHA, 38 received a sulphonylurea, 32 a biguanide and 31 a sulphonylurea and biguanide combination. All patients attended the Diabetic and Dietetic Department, Royal Infirmary, Edinburgh, and none had previously been suspected of suffering from thyroid disease.

Serum total thyroxine (T4) and TSH concentrations were estimated in all patients. The sera of 171 insulin-dependent diabetics, 96 patients treated with OHA and 55 treated with diet alone, including all those patients with a raised serum TSH, were examined for antibodies to thyroid microsomes and thyroglobulin.

Serum T4 was measured by radio-immunoassay (Seth et al 1974) the inter-assay co-efficient of variation using anonymous control sera averaged 11.7%. The reference (normal) range for serum T4 was 60-150nmol/l for both males and females. Serum TSH was measured by double antibody

radio-immunoassay using IRP 68/38 as standard (Toft et al., 1978). The between assay co-efficient of variation was 5.1%. In euthyroid patients attending an Endocrine Clinic, the 95th percentile of TSH concentration was 5.7mU/l which was therefore regarded as the upper limit of the reference (normal) range, with 5% of patients having concentrations between 5.7 - 10mU/l.

Antibodies to thyroid microsomes were measured by the Fujizoki tanned cell haemagglutination technique (Amino et al., 1976) in which a titre of $1/160^2$ was considered positive. Antibodies to thyroglobulin were also measured by a tanned cell haemagglutination technique in which a titre of $1/25$ was regarded as positive.

Statistical analysis was by Student's t test unless otherwise stated.

Results

An elevated serum TSH concentration was found in 24 insulin-dependent diabetics, eight diabetics treated with OHA and two treated with diet alone. The prevalence of a raised serum TSH concentration in insulin-dependent diabetics (13%) was twice that (6.1%) of non-insulin-dependent diabetics. The mean \pm SE serum TSH concentrations in patients whose concentrations were elevated and normal were 26.6 ± 7.1 mU/l and 2.2 ± 1.1 mU/l, respectively. Of the 34 patients with an elevated serum TSH concentration, nine (26.5%) had serum T4 concentrations below the normal range (mean 44.1 ± 3.7 nmol/l). The mean serum T4 concentration of the remaining patients with a raised serum TSH was 91.0 ± 4.2 nmol/l which was significantly lower ($p < 0.01$) than the level of $104.0 \pm$

1.2nmol/l found in patients with normal serum TSH concentrations.

The mean ages at diagnosis of diabetes (37.5 ± 2.8 years) and at time of study (55.8 ± 2.3 years) of insulin-dependent diabetics with a raised serum TSH concentration were greater ($p < 0.02$ and < 0.01) than the corresponding ages (30.4 ± 1.1 and 47.8 ± 1.1 years) of insulin-dependent diabetics with a normal TSH concentration. The duration of diabetes (17.8 ± 1.6 years) of insulin-dependent diabetics with a raised serum TSH concentration was similar to that (17.4 ± 0.7 years) of insulin-dependent diabetics with a normal serum TSH concentration. In particular, for a given age at diagnosis, or a given age at the time of study, there was no difference in the duration of diabetes when comparing insulin-dependent diabetics with a raised and those with a normal serum TSH concentration. This was investigated by stratifying the patients according to age in decades and, within each age group, calculating the difference in mean duration of diabetes in insulin-dependent diabetics with normal or elevated serum TSH concentrations, together with the variance of this difference. The results from all age groups were then amalgamated into an overall test by forming a weighted mean difference in duration of diabetes (weights being chosen inversely proportional to the variances) and calculating the standard error of this weighted mean. The significance was then determined by referring the ratio of mean difference to its standard error to tables of the t-distribution. The stratification procedure was necessary to remove the effect of the inevitable association between the age of the patient and the duration of diabetes.

In contrast to the insulin-dependent diabetics, age at time of

study did not significantly influence the prevalence of a raised TSH concentration in non-insulin-dependent diabetics. Thus, of 52 such patients aged less than 50 years, 4 (7%) had an elevated TSH concentration, whereas 6 (6%) of 109 patients aged 50 years or more had an elevated TSH concentration. Within the OHA treated patients, there was no difference in the frequency with which a raised serum TSH concentration was found among patients treated with a sulphonylurea, a biguanide or a combination of both drugs.

No patient had an elevated serum T₄ concentration.

The prevalences of thyroid microsomal and thyroglobulin antibodies in patients with raised and with normal serum TSH concentrations grouped according to treatment are shown in Table 3. It was possible to demonstrate a significantly higher prevalence of thyroid microsomal antibodies, but not thyroglobulin antibodies, in insulin-dependent diabetics and OHA treated diabetics with raised TSH levels than in those with normal TSH concentrations, by means of the χ^2 -squared test with Yates' correction ($p < 0.0005$ and $p < 0.0005$, respectively). There was no significant difference in the prevalence of thyroid microsomal antibodies between insulin-dependent diabetics and OHA treated diabetics with raised serum TSH concentrations. The number of patients treated with diet alone who had thyroid antibodies was too small for statistical analysis.

Discussion

The prevalence of a raised TSH concentration in the non-insulin-dependent diabetics (6.6%) described in this study is similar to that

reported in a non-diabetic population (5%) by Tunbridge et al (1977a). On the other hand, a raised serum TSH concentration is twice as common in insulin-dependent diabetics (13%) as is found in non-insulin-dependent diabetics or non-diabetic controls. These findings are in accordance with the distribution of thyroid antibodies reported in the diabetic population of whom only insulin-dependent diabetics exhibit a higher prevalence than controls (Landing et al., 1963; Irvine et al., 1970; Whittingham et al., 1971; Neufeld et al., 1980). Moreover, the present study reports a clear association between the presence of thyroid microsomal antibodies and a raised serum TSH concentration in insulin and OHA treated diabetics. Thyroglobulin antibodies are also more commonly found in insulin and OHA treated diabetics with raised than with normal TSH concentrations but the difference fell short of statistical significance. The association between thyroid failure and thyroid antibodies suggests that the underlying thyroid pathology is that of autoimmune thyroiditis. It is of interest that the reported antithyroid effect of the sulphonylureas (Hunton et al., 1965) was not sufficient to be manifest as thyroid failure in the present study. Prince et al (1980) came to the same conclusion having studied thyroid function and antibody tests in a group of diabetics of differing treatment categories, of whom only 2 (5%) of 41 patients receiving sulphonylureas had raised serum TSH concentrations.

In contrast to the apparent association between insulin-dependent diabetes, thyroid microsomal antibodies and a raised serum TSH concentration, Feely and Isles (1979) have reported minimal elevation of the TSH concentration in a large proportion (38%) of non-insulin-dependent diabetics, most of whom were thyroid antibody negative. The upper limit

of their normal range for serum TSH concentration was based on values obtained from control subjects younger than the diabetics under consideration and the significance of a raised TSH concentration of between 4.5 - 6.0mU/l is questionable. Moreover, patients who had previously been treated for hyperthyroidism were included in their cross-sectional survey of thyroid function. Elevation of the serum TSH concentration following surgical or irradiation treatment of hyperthyroidism is not uncommon and the inclusion of such patients will inevitably distort the apparent distribution of primary thyroid failure in the diabetic population.

Clearly only a proportion of insulin-dependent diabetics are subject to the development of coincident thyroiditis and thyroid failure, the risk apparently increasing with increasing age at time of study and age at time of diagnosis of diabetes. A cross-sectional study, such as this, is subject to selective mortality of groups of patients such as has been proposed (Whittingham et al., 1971) for insulin-dependent diabetics with circulating thyro-gastric antibodies. Had this been a major influence, one would have expected duration of diabetes in patients with an elevated serum TSH concentration to be shorter than that of euthyroid diabetics. However, durations of diabetes were identical in both groups of insulin-dependent diabetics which also indicates that the state of carbohydrate intolerance cannot, per se, account for the development of a raised serum TSH concentration.

Although the excessive prevalence of thyroid microsomal antibodies in diabetics is most evident in young insulin-dependent diabetics (Irvine et al., 1970; Whittingham et al., 1971), this group appears

not to be at immediate risk of developing thyroid failure. Instead, the older insulin-dependent diabetics are at greatest risk of primary thyroid failure, manifest as a raised serum TSH concentration. It is tempting to suppose that thyroid failure may only develop after a prolonged period of autoimmune thyroiditis accompanied by circulating thyroid microsomal antibodies. It may be seen (Table 3) that 19% of insulin-dependent diabetics with a normal serum TSH concentration have thyroid microsomal antibodies and such patients may be at risk of developing thyroid failure in future.

TABLE 3

Prevalence of thyroid autoantibodies in the sera of diabetic patients according to the type of treatment and serum TSH level

Treatment	Serum TSH	No. of patients	Microsomal antibody positive		Thyroglobulin antibody positive		Significance
			No.	%	No.	%	
Insulin	raised	24	21	88	5	21	n.s
	normal	147	28	19	12	8	
OHA	raised	8	5	63	1	13	n.s
	normal	88	4	5	5	6	
Diet	raised	2	1	50	0	0	-
	normal	53	4	7	3	6	

CHAPTER 4

Distribution of unrecognised primary thyroid failure in insulin-dependent diabetics.

It has been shown in the previous chapter that the risk of a raised serum TSH concentration parallels that of circulating thyroid antibodies, being greater in insulin-dependent diabetics than in non-insulin-dependent diabetics and controls, in whom the risks are not significantly different. Within the population of insulin-dependent diabetics only a proportion of patients appear to develop autoimmune thyroiditis but the characteristics of those individuals predisposed to coincident thyroid disease remain ill defined. It was therefore decided to examine a larger group of insulin-dependent diabetics in order to establish which patients are most at risk of clinically unrecognised primary thyroid failure in terms of sex, age at diagnosis of diabetes, age at time of study and duration of diabetes.

Subjects and Methods

Six hundred and five insulin-dependent diabetics (294 males and 311 females) aged 21-84 years, who were attending the Diabetic and Dietetic Department, Royal Infirmary, Edinburgh, and in whom thyroid disease had not previously been suspected were studied. Table 4 shows the distribution of these patients according to sex, age at time of study and age at diagnosis of diabetes. Patients were categorized according to whether they were treated with insulin at or within three months of diagnosis ("primary" insulin-dependent diabetics), or were treated with insulin more than three months from diagnosis after exhibiting secondary sulpho-

nylurea failure ("secondary" insulin-dependent diabetics). The serum TSH concentration was measured in all patients and in those having a raised TSH level, the serum T4 concentration was also estimated.

The serum T4 and TSH concentrations were measured by previously described radio-immunoassays (Seth et al., 1976; Toft et al., 1978).

The statistical analyses were carried out by fitting a linear logistic or log linear model to the data using the GLIM computer package, unless otherwise indicated.

Results

Seventy-one (11.7%) of the insulin-dependent diabetics studied had elevated serum TSH concentrations and 15 (21%) of these had serum T4 concentrations below the normal range. Figure 1 shows the distribution of serum T4 and TSH concentrations in the patients with primary thyroid failure.

Figure 2 shows the prevalence of an elevated serum TSH concentration in male and female insulin-dependent diabetics according to age at diagnosis of diabetes and age at the time of study. The prevalence of a raised serum TSH concentration in females of all ages (17.0%) was significantly greater ($p < 0.0005$) than that in males (6.1%). The prevalence of a raised serum TSH concentration increased with increasing age at onset of diabetes ($p < 0.05$) and age at time of the study ($p < 0.001$) in females but not in males. Thus, of 156 females aged 50 years or more at the time of the study, 38 (24.4%) had elevated serum TSH concentrations, and 8 (5.1%) of these had low serum T4 concentrations. Of 138 males aged 50 years or more at the time of the study, 11 (8%) had elevated

serum TSH concentrations and 4 (2.9%) had low serum T4 concentrations.

The duration of diabetes of 17.7 ± 1.3 years in insulin-dependent diabetics with raised serum TSH concentrations is not significantly different (Students t test) from that of 15.5 ± 0.4 years in insulin-dependent diabetics with normal serum TSH concentrations. There was no difference in the duration of diabetes for any given age at diagnosis of diabetes or any given age at time of study when comparing insulin-dependent diabetics with raised and with normal serum TSH concentrations. Similarly, no difference was observed when male and female insulin-dependent diabetics were considered independently.

In females aged 40 years or more at the time of study, it was not possible to establish a statistically significant difference between the prevalence of a raised serum TSH concentration in "primary" insulin-dependent diabetics (20%) and that in "secondary" insulin-dependent diabetics (23.4%). Similarly, in males aged 40 years or more at the time of study, the prevalence of a raised serum TSH concentration was not significantly higher in "primary" insulin-dependent diabetics (8.4%) than in "secondary" insulin-dependent diabetics (5.7%).

Discussion

This study confirms the risk of unrecognised or subclinical thyroid failure in insulin-dependent diabetics to be approximately twice that found in non-insulin-dependent diabetics and non-diabetic controls. In addition, it appears that the increased risk is found mainly in women of whom almost one in five has an elevated serum TSH concentration. Nevertheless, the prevalence of subclinical thyroid failure in male

insulin-dependent diabetics is also twice that reported in male non-diabetics (Tunbridge et al., 1977a). The particular susceptibility of the older, female insulin-dependent diabetics to primary thyroid failure accords with the reported distribution of thyroid microsomal antibodies in the diabetic population (Irvine et al., 1970; Whittingham et al., 1971). Female proponderance is also recognised in other autoimmune diseases appearing in middle age such as pernicious anaemia, Addison's disease, primary atrophic hypothyroidism, Graves' disease and Hashimoto's thyroiditis.

In female insulin-dependent diabetics, the frequency with which a raised serum TSH concentration is found increases with increasing age at onset of diabetes and with increasing age at time of study, which are inter-dependent. If increasing age at diagnosis of diabetes was the main influence on the risk of developing primary thyroid failure, then insulin-dependent diabetics with raised serum TSH concentrations would have been diabetic for less time for any given age at the time of study, than insulin-dependent diabetics with normal serum TSH concentrations. If senescence alone increased the risk of developing primary thyroid failure, then insulin-dependent diabetics with raised serum TSH concentrations would have been diabetic longer for any given age at diagnosis of diabetes than diabetics with normal serum TSH concentrations. However, it was found that the duration of diabetes was similar in diabetics with raised and normal serum TSH concentrations and therefore, it was not possible to determine whether age at diagnosis of diabetes or age at time of study was the predominant influence on the probability of finding a raised serum TSH concentration. It has been shown (Chapter 6) that there is a correlation between age at onset of diabetes and that of

thyroid failure, such that age at study and age at onset of diabetes may equally determine the risk of developing thyroid failure.

Little attempt has yet been made to compare late-onset "primary" and "secondary" insulin-dependent diabetics. Although they are both regarded as type 1 diabetics, having the same aetiology as the more typical juvenile onset insulin-dependent diabetics, confirmation in terms of immunological and genetic studies is lacking. The present study shows no difference in the prevalence of associated thyroid failure between late-onset "primary" and "secondary" insulin-dependent diabetics, and to this extent the two groups are similar. It seems likely, however, that the two groups differ in the rate of deterioration of carbohydrate tolerance, since some "secondary" insulin-dependent diabetics remain insulin independent for many years after the diagnosis of diabetes.

Conclusion

These studies show that unrecognised primary thyroid failure is extremely common in the insulin-dependent diabetic population, and is associated with circulating thyroid microsomal antibodies indicative of underlying autoimmune thyroiditis. The risk is greatest in females, increases with increasing age, and is similar in patients requiring insulin treatment from the outset and those exhibiting secondary sulphonylurea failure. It may be seen that the same diabetic patients are particularly susceptible to overt as to unrecognised primary thyroid failure.

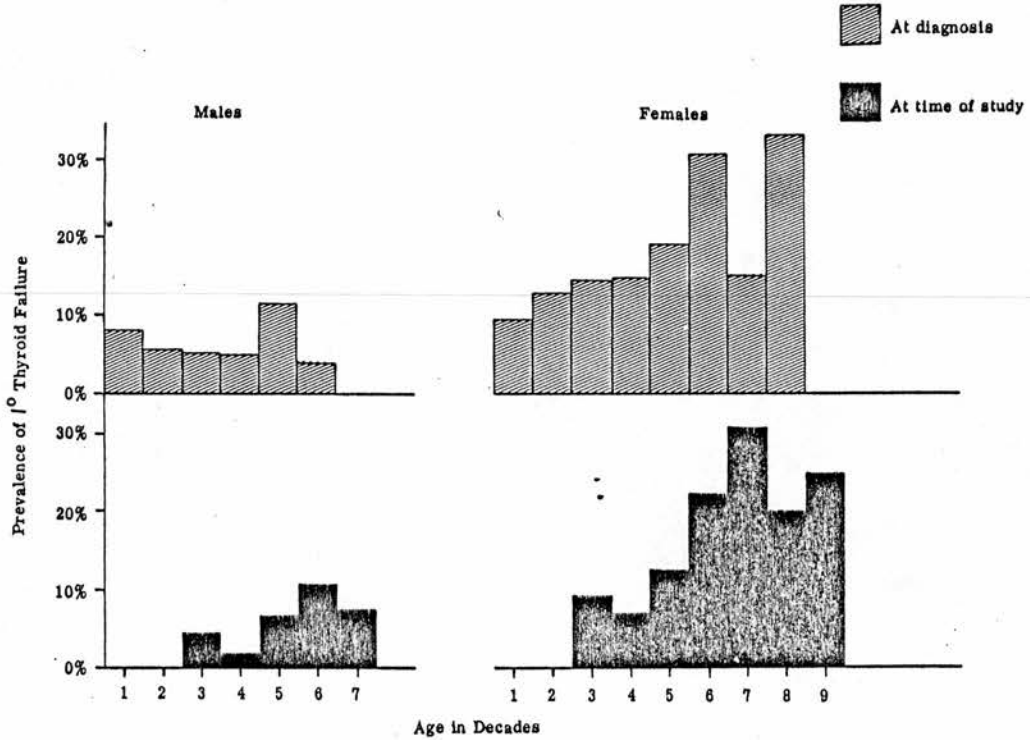


Fig. 2

Prevalence of an elevated TSH concentration in insulin-dependent diabetics according to sex, age at diagnosis of diabetes, and age at time of study.

TABLE 4

Sex and age of 605 insulin-dependent diabetics at diagnosis of diabetes and at time of study

	Age (yr)								
	0-	10-	20-	30-	40-	50-	60-	70-	80-
<u>At diagnosis</u>									
Females	21(0)	55(0)	70(0)	61(10)	37(57)	41(56)	20(60)	6(67)	
Males	12(0)	52(0)	78(1)	60(17)	52(40)	25(60)	14(57)	1(100)	
<u>At time of study</u>									
Females			54(0)	45(2)	56(14)	67(48)	41(41)	40(40)	8(50)
Males			45(0)	53(6)	58(12)	65(26)	54(33)	19(58)	

The percentage of "secondary" insulin-dependent diabetes is shown in parenthesis

CHAPTER 5

Natural history of thyroid function in diabetics with impaired thyroid reserve, including an examination of the lipid profiles of diabetics with primary thyroid failure and their response to thyroxine treatment.

The prevalence of impaired thyroid reserve, as evidenced by a raised serum TSH concentration but serum T4 concentration within the normal range, is increased in insulin-dependent diabetics with older female patients being particularly at risk. Although it seems likely that thyroid failure develops only after prolonged thyroiditis, the natural history of thyroid function in such diabetics has not yet been studied. Cross-sectional studies suffer from the possible selective mortality of groups of subjects such as those with thyroid antibodies (Whittingham et al., 1971). Studies of the natural history of autoimmune thyroiditis in non-diabetics are not uniform (Gordin and Lamberg, 1981; Tunbridge et al., 1981) and may bear little relation to the natural history of this condition in the diabetic population. Therefore, a group of diabetics with impaired thyroid reserve has been studied prospectively and the natural history of thyroid function in these patients has been compared with that of a group of diabetics having normal thyroid function. Mortality in these two groups of patients has also been compared with that of diabetics found to be hypothyroid and treated with thyroxine.

Patients and Methods

Patients comprised those described in previous cross-sectional studies of the prevalence of unrecognised thyroid failure in the diabetic

population, carried out between 1973-1979 (Chapters 3 and 4). Eighty patients had elevated serum TSH concentrations (median 8.9mU/l, range 5.8-46.3mU/l) but serum T4 concentrations (median 82.0nmol/l, range 60-136nmol/l) within the normal range (Group 1). Thyroid function and antibody status of these patients were reviewed, where possible, at regular intervals (1-3 years) until 1981. Patients were only treated with thyroxine when found to have subnormal T4 and elevated TSH concentrations in the serum. Fifty nine patients in Group 1 completed review and were individually matched retrospectively for age, sex, duration of diabetes and diabetic treatment with diabetics having had normal serum T4 and TSH concentrations during the original cross-sectional studies (Group 2). A single review of thyroid function and antibody status was attempted for each control subject of Group 2 in 1981. Group 3 included 24 patients found between 1973-1979 to have subnormal serum T4 concentrations (median 42nmol/l, range 25-59nmol/l) and elevated serum TSH concentrations (median 37mU/l, range 10-239mU/l), who were thereafter treated with thyroxine in a dose of 0.1 - 0.25mgs daily, sufficient to maintain the serum TSH concentration within the normal range and the patient clinically euthyroid.

Table 5 shows the sex, age, duration of diabetes and treatment of patients in the three groups when first recruited.

The serum T4 and TSH concentrations were measured by previously described radio-immunoassays (Seth et al., 1976; Toft et al., 1978).

Antibodies to thyroid microsomal antigen were measured by the indirect immuno-fluorescence technique using undiluted serum. Antibodies to thyroglobulin were detected by means of the tanned cell

haemagglutination technique, taking a titre of ≥ 25 as significant. Results are expressed as median and range or mean \pm standard error (SE), where appropriate. Statistics were carried out by means of χ^2 analysis and Student's t test.

Results

The mean periods of follow-up from recruitment until 1981 were similar for patients in Group 1 (4.2 ± 0.2 years), Group 2 (4.2 ± 0.2 years) and Group 3 (4.0 ± 0.3 years). Table 6 shows the numbers of patients in each group who successfully completed follow-up and those who were lost to follow-up or died. Similar proportions of patients in the three groups were lost to follow-up or successfully completed full review. Although fewer patients in Group 2 died (12%) than those in Group 1 (20%) and Group 3 (25%) these differences were not statistically significant.

Thyroid Function

Fifty-nine patients in Group 1 successfully completed follow-up until 1981, each having been reviewed 1-5 times, the majority (85%) having been reviewed twice or more over a period of 2-8 years. The natural history of thyroid function in these patients exhibited four different patterns:

- (a) All subsequent serum TSH and T4 concentrations falling within the normal range (n = 7)
- (b) Intermittent elevation of the serum TSH concentration with serum T4 concentrations remaining within the normal range (n = 14)

- (c) Persistent elevation of the serum TSH concentration with serum T4 concentrations remaining within the normal range (n = 29)
- (d) Persistent elevation of the serum TSH concentration with serum T4 concentrations falling below the normal range (n = 9)

Table 7 shows the median serum TSH and T4 concentrations at the time of recruitment and in 1981 (or when first found to have subnormal serum T4 concentrations) of the Group 1 patients according to their natural history of thyroid function. It may be seen that of the 59 patients followed for approximately 4 years, 9 (3.6% per annum) became hypothyroid and were treated accordingly. The majority (86%) of the remaining patients continued to exhibit impaired thyroid reserve without significant change in thyroid function, including 14 patients (Group 1b) who showed intermittent elevation of serum TSH concentration, whose sequential serum TSH and T4 concentrations are shown in Table 8. The minority (Group 1a; 14%) regained normal thyroid function. It was not possible to predict the natural history of thyroid function in individual patients on the basis of their initial serum T4 and TSH concentrations.

At the time of recruitment, median serum T4 and TSH concentrations in patients of Group 2 were 99nmol/l (range 68-148nmol/l) and 1.9mU/l (range 0.8 - 4.7mU/l) and had not significantly changed in 47 patients retested in 1981 (94nmol/l, range 60-132nmol/l and 1.9mU/l, range 1.0 - 6.2mU/l). Included within the 47 patients who were reviewed in 1981 were two female patients with minimally elevated TSH concentrations (6.1 and 6.2 mU/l) but normal serum T4 concentrations (84 and 80 nmol/l). The remaining 45 patients had normal thyroid function.

Thyroid Antibody Status.

At recruitment, 52 (65%) patients of Group 1 and 9(15%) of Group 2 had positive thyroid microsomal antibodies ($p < 0.01$), whereas at the same stage 14 (18%) of Group 1 and 1 (2%) of Group 2 had positive thyroglobulin antibodies. Table 7 shows the thyroid antibody status of patients in Group 1 who completed follow-up in relation to natural history of thyroid function. Positive thyroid microsomal antibodies tended to be associated with development of hypothyroidism and patients who subsequently exhibited normal thyroid function were commonly negative for thyroid microsomal antibodies. However, there was no significant difference between the Group 1 patients showing the four different types of natural history in respect of prevalence of thyroid microsomal antibody at recruitment. Similarly, although the proportion (20.5%) of thyroid microsomal antibody positive Group 1 diabetics (n 39) who developed hypothyroidism was greater than that (5%) of antibody negative Group 1 diabetics (n 20), this difference was not statistically significant.

At follow-up, 4 (7%) patients in Group 2 had thyroid microsomal antibodies and 3 (6%) had thyroglobulin antibodies. Of the two patients in Group 2 who developed an elevated serum TSH concentration, 1 had positive thyroid microsomal antibodies at recruitment and at follow-up, and positive thyroglobulin antibodies at follow-up only. The other patient remained thyroid antibody negative.

Table 9 compares the thyroid microsomal antibody status at recruitment and at follow-up, in 1981, of patients in Group 1 and 2. Similar proportions of patients in Group 1 (85%) and Group 2 (82%) maintained their antibody status during the study. However, the majority (78%) of



patients in Group 2 who were initially antibody positive subsequently became antibody negative.

DISCUSSION

Although insulin-dependent diabetics are susceptible to impaired thyroid reserve it cannot be assumed that all such affected patients invariably develop frank hypothyroidism. This study shows that only a proportion of diabetics with impaired thyroid reserve demonstrate progressive thyroid failure to hypothyroidism. Those with elevated serum TSH concentrations accompanying thyroid microsomal antibodies are at greatest risk of developing hypothyroidism (5% per annum), whereas, patients with elevated serum TSH concentration without thyroid microsomal antibodies rarely develop hypothyroidism (1% per annum). A similar experience was reported by Tunbridge et al. (1981) who observed hypothyroidism to develop at a rate of 5% per annum in non-diabetics with elevated TSH concentrations and positive thyroid microsomal antibodies. Thus, the coincidence of diabetes has no bearing on the natural history of autoimmune thyroiditis. A higher incidence of hypothyroidism (26% per annum) is reported (Gordin and Lamberg, 1981) in non-diabetic patients with symptomless autoimmune thyroiditis who had substantially higher initial TSH concentrations, as might be predicted.

The presence of thyroid microsomal antibodies without elevation of the serum TSH concentration appears to have little predictive value, such patients commonly losing the antibody at review. Transient appearance of thyroid antibodies (Hawkins et al., 1980; Tunbridge et al., 1981) may be associated with intercurrent viral illnesses which probably have little bearing on long-term thyroid function. Nevertheless it has

been suggested (Riley et al., 1981) that positive thyroid microsomal antibodies alone are sufficient grounds for annual review of thyroid function. In our experience, this is not necessary since thyroid function deteriorates very slowly, if at all, in such patients, many of whom exhibit thyroid microsomal antibodies transiently. Similarly, Gordin and Lamberg (1981) report that of ten non-diabetic patients with symptomless autoimmune thyroiditis, but normal serum TSH concentrations, who were followed approximately five years, none developed hypothyroidism. Although patients at greatest risk of developing hypothyroidism are those with thyroid microsomal antibodies and elevated serum TSH concentrations, not all such patients demonstrate progressive thyroid failure and it was not possible to predict the outcome in individual patients using the indices of this study. A variable degree of thyroid underactivity, as evidenced by intermittent elevation of serum TSH concentrations, is exhibited by a substantial proportion of patients which further complicates any attempt to predict future thyroid dysfunction. Transient elevation of serum TSH concentration has been noted in non-diabetics, but may be yet more common in diabetics where metabolic control may influence the prevailing serum TSH concentration (Alexander et al., 1982).

In contrast to the general agreement regarding which subjects with impaired thyroid reserve are at greatest risk of developing hypothyroidism, there is widespread uncertainty as regards their appropriate management. Although the mortality of diabetics with impaired thyroid reserve is approximately twice that of euthyroid diabetics, the difference was not significant. A significant difference might have helped to explain the disproportionate cardiovascular mortality of middle-aged

female insulin-dependent diabetics (Garcia et al., 1974; Gordon et al., 1977) - the group we have shown to be most at risk of sub-clinical thyroid failure. It is possible that treatment with thyroxine supplements of all patients with elevated TSH concentrations and thyroid microsomal antibodies might improve their survival and although this policy has been advocated (Tunbridge et al., 1981), it remains of no proven value. Thyroxine treatment of a small group of hypothyroid patients failed to appreciably improve their mortality which remained similar to that of diabetics with impaired thyroid reserve. It could be argued that significant atherosclerosis had developed prior to institution of thyroxine treatment and that such treatment under these circumstances might only precipitate myocardial infarction. Whether or not diabetics with impaired thyroid reserve are treated with thyroxine, they will require regular review of thyroid function, with dose adjustment or initiation of thyroxine therapy, accordingly.

Plasma lipids in diabetics with primary thyroid failure

We have shown that the mortality of diabetics with untreated impaired thyroid reserve or those who have been treated with thyroxine for hypo thyroidism, is approximately twice that of euthyroid diabetics. The difference in mortality was not significant, possibly due to the relatively small number of patients under review. Indirect support for the hypothesis that diabetics with thyroiditis are at particular risk of premature cardiovascular disease has previously been provided by the cross-sectional study of Whittingham et al (1971). Similarly, non-diabetics with symptomless autoimmune thyroiditis have been described (Bastenie et al., 1971; 1977; Tunbridge et al., 1977b) to be at

increased risk of cardiovascular disease. One potential consequence of primary thyroid failure, known to be a risk factor for cardiovascular disease in the diabetic population (Lowy and Barach, 1958), is hypercholesterolaemia. While hypercholesterolaemia is recognised to be a feature of hypothyroidism (Peters and Man, 1950; O'Hara et al., 1966), the lipid profile of patients with impaired thyroid reserve is controversial (Bastenie et al., 1971; 1977; Tunbridge et al., 1977b). The lipid profile of diabetics with primary thyroid failure has not yet been examined and is the subject of this study.

Patients and Methods.

Forty-nine patients (Group 1: 11 males, 38 females) with raised serum TSH concentrations and 49 patients (Group 2: 12 males, 37 females) with normal serum TSH and T₄ concentrations were studied. The mean age, duration of diabetes and per cent ideal body weight of patients in Group 1 were 55.5 ± 1.6 years, 16.4 ± 1.2 years and $100.6 \pm 1.8\%$ and of patients in Group 2 were 59.3 ± 1.5 years, 16.8 ± 1.2 years and $95.2 \pm 1.6\%$ ideal body weight. Forty-two patients in Group 1 and 44 patients in Group 2 were insulin treated, the remaining patients receiving a carbohydrate restricted diet with or without OHA therapy. For the purpose of further analysis, patients in Group 1 were divided into those having subnormal serum T₄ concentrations (Group 1a, n 22) and those whose serum T₄ concentrations were within the normal range (Group 1b, n 27). None received drugs known to alter plasma lipids and diabetic control was satisfactory in each patient, who was free of ketonuria on the morning of the study. Three patients in Group 1 and five in Group 2 had elevated serum creatinine concentrations (150-200 $\mu\text{mol/l}$) although none

was nephrotic.

Fasting venous blood samples were obtained and analysed for cholesterol, triglycerides and lipoproteins together with TSH and T4.

Plasma cholesterol and triglyceride estimations were carried out on a Technicon SMAC (Technicon Instruments Company, Basingstoke, England) using standard methodology and reagents provided for this instrument by the manufacturers. Between-batch precision was 2.5% coefficient of variation for cholesterol and 5% coefficient of variation for triglyceride. In our laboratory, the normal range for adults for plasma cholesterol is 3.6 - 6.7mmol/l and for plasma triglyceride is 0.6 - 1.7 mmol/l. Lipoprotein electrophoresis was carried out on agarose (Corning ACI, Palo Alto, USA), and the stained strips were assessed visually.

Statistical analysis was carried out by Student's t test and Wilcoxon's test for paired differences.

Results

Table 10 compares plasma lipids and mean T4 and TSH concentrations of patients in Groups 1 and 2. The mean plasma cholesterol concentration is higher in patients of Group 1a ($p < 0.005$) and higher in all patients of Group 1 ($p < 0.05$) than in patients of Group 2. There was no significant difference in the mean cholesterol concentration of patients in Group 1b and that of patients in Group 2. Patients of Groups 1 and 2 had similar triglyceride concentrations.

A significant relationship was observed between plasma cholesterol and serum TSH concentration ($r = 0.56$, $p < 0.01$) and serum T4 concentra-

tions ($r = -0.51$, $p < 0.05$) in respect of patients in Group 1a, but not in patients of Group 1b nor patients in Group 2.

In 18 patients of Group 1a, six weeks thyroxine replacement therapy (0.1 - 0.2 mg daily) led to a significant reduction ($p < 0.01$) in mean plasma cholesterol concentration to 5.8 ± 0.3 mmol/l and a non-significant reduction in mean triglyceride concentration to 1.33 ± 0.2 mmol/l while returning thyroid function to normal (TSH 2.74 ± 0.4 mU/l, T4 96.7 ± 5.2 nmol/l). Four of seven patients with Type 2 hyperlipoproteinaemia regained a normal lipoprotein electrophoretic pattern.

Discussion

This study shows that clinically unrecognised primary thyroid failure in diabetics is associated with a modest, yet significant, elevation of the plasma cholesterol concentration. The elevation is only significant in hypothyroid diabetics who show a correlation between degree of thyroid underactivity and degree of hypercholesterolaemia, and in whom hypercholesterolaemia is reversed by thyroxine treatment. Diabetics with elevated serum TSH concentrations but serum T4 concentrations within the normal range are not hypercholesterolaemic. These findings are in accordance with those reported in non-diabetics with primary thyroid failure of whom only the hypothyroid subjects demonstrate hypercholesterolaemia (Bastenie et al., 1971; Tunbridge et al., 1977b; Kutty et al., 1978). Reports of hypercholesterolaemia in the presence of an exaggerated TRH response or minimal elevation of basal TSH concentration only (Alaghband-Zadeh et al., 1977), have not been confirmed and thyroxine treatment of such patients does not alter the plasma cholesterol concentration (Nilsson et al., 1976).

If macrovascular disease is a feature of diabetics having impaired thyroid reserve and autoimmune thyroiditis, the macroangiopathy cannot be attributed to hypercholesterolaemia. It remains possible that circulating thyrogastric antibodies may be atherogenic as suggested by Mathews et al.(1974).

TABLE 5

Clinical characteristics of study groups at recruitment

Group	Sex	Age (yr)	Duration of diabetes (yr)	<u>Diabetic treatment</u>		
				Insulin	Oral hypoglycaemic agent	Diet
1	65F, 15M	55.1 ± 1.6	15.1 ± 1.2	65	12	3
2	48F, 11M	56.5 ± 1.9	15.5 ± 1.3	46	10	3
3	18F, 6M	56.5 ± 2.5	15.4 ± 2.1	20	2	2

Data expressed as mean ± SE

TABLE 6

Outcome of study groups after four year follow-up

Group	Completed follow-up	Lost to follow-up	Died
1 n=80	59(74%)	5(6%)	16(20%)
2 n=59	47(80%)	6(10%)	7(12%)
3 n=24	16(67%)	2(8%)	6(25%)

TABLE 7

Four year natural history of thyroid function in diabetics

with impaired thyroid reserve (Group 1)

Natural History [†]	TSH* (mU/l)	T4* (nmol/l)	TM (% positive)	TG (% positive)
(a) n = 7	Initial (6.0 - 18.5)	100 (64 - 130)	29	14
	Final (2.7 - 4.9)	95 (62 - 112)	29	0
(b) n = 14	Initial (5.8 - 20)	83 (63 - 136)	43	21
	Final (4.2 - 17.8)	79 (60 - 102)	43	14
(c) n = 29	Initial (5.9 - 34)	81 (60 - 130)	79	14
	Final (5.8 - 31.5)	75 (60 - 128)	72	21
(d) n = 9	Initial (5.8 - 46.3)	80 (60 - 101)	89	22
	Final (15.5 - 69.1)	54 (36 - 59)	78	22

* values expressed as medians with range in parenthesis
 TM: thyroid microsomal antibodies. TG: thyroglobulin antibodies

† see text for explanation

TABLE 8

Thyroid function in diabetics with intermittent elevation of serum TSH concentration

Patient		Year of follow-up								
		0	1	2	3	4	5	6	7	8
1	TSH(mU/l)	9.5				3.2		6.7		10.4
	T ₄ (nmol/l)	111				93		99		78
2		10.0	6.6	4.7						
		96	70	102						
3		7.5	4.3	5.7	5.3		6.5			
		136	113	112	110		96			
4		8.0	14.9	0.7	5.2	9.9				
		83	71	131	81	87				
5		8.8	8.4		5.4					
		69	76		81					
6		5.9	9.4		4.5					
		85	86		99					
7		5.8	4.9		6.3					
		78	80		78					
8		9.3	5.9	5.2	4.2					
		63	67	66	60					
9		8.9	4.9			17.8				
		117	100			79				
10		6.8	6.4	4.5						
		91	125	96						
11		7.2			5.5			8.5		5.0
		68			76			73		63
12		6.8				7.0	3.7			4.9
		117				106	88			78
13		20.0	6.7		5.2					
		70	103		94					
14		6.4	9.7	7.8			5.5			
		76	69	79			63			

TABLE 9

Natural history of thyroid microsomal antibody status

Thyroid Microsomal Antibody
Status at Follow-up (1981)

	Positive		Negative		Total	
	Gp1	Gp2	Gp1	Gp2	Gp1	Gp2
Initial thyroid microsomal antibody status						
Positive	33	2	6	7	39	9
Negative	3	2	17	38	20	40
Total	36	4	23	45	59	49

TABLE 10

Comparison of thyroid function and plasma lipids in patients of Group 1 and Group 2

Group	T ₄ (nmol/l)	TSH (mU/l)	Cholesterol (mmol/l)	Triglyceride (mmol/l)	Lipoprotein Electrophoretic pattern	
					Normal	Type II Type IV
1a	n=22 38.6 ± 3.3	51.6 ± 6.4	7.2 ± 0.4	1.37 ± 0.1	12	9 1
1b	n=27 83.0 ± 3.7	20.3 ± 3.3	6.4 ± 0.3	1.33 ± 0.2	16	9 2
1a + 1b	n=49 63.0 ± 4.0	38.0 ± 5.4	6.8 ± 0.2	1.35 ± 0.1	28	18 3
2	n=49 99.1 ± 2.8	3.16 ± 0.2	6.0 ± 0.2	1.18 ± 0.1	39	10 0

Data expressed as mean ± SE

PART 3

CHAPTER 6

The clinical features of diabetics with coexisting autoimmune thyroid disease.

It has been shown earlier in the thesis (Chapters 2, 3 and 4) that clinically evident autoimmune hyper- and hypothyroidism together with clinically unrecognised primary thyroid failure are found more frequently in the insulin-dependent diabetic population than in controls and non-insulin-dependent diabetics. A study of a large group of diabetics with coexisting, clinically evident autoimmune thyroid disease whose clinical characteristics may be compared and contrasted with subjects having diabetes or thyroid disease alone was undertaken.

Patients and Methods

Two hundred and fifteen diabetics with associated overt thyroid disease were studied who had been randomly selected from patients attending the Diabetic and Dietetic Department, Royal Infirmary, Edinburgh. Although they represented every recognised case of coexisting autoimmune thyroid disease and diabetes, the possibility remains that a number of such patients within the clinic population failed to be identified and were therefore inadvertently omitted. Where hypothyroidism followed treatment of hyperthyroidism, the patient was classified in the hyperthyroid group only. The clinical diagnoses of hyperthyroidism and primary hypothyroidism were confirmed by currently available tests of thyroid function, including estimation of plasma protein - bound iodine, effective thyroxine ratio, T4 and TSH concentrations and by means of the

TSH stimulation test. Hyperthyroidism was shown to be due to Graves' disease on the basis of clinical, histological and/or radio-isotope scanning evidence whereby patients with an overactive nodular goitre or other cause of non-Graves' hyperthyroidism were excluded. In the population of diabetics under consideration, the aetiology of primary hypothyroidism may be assumed to be autoimmune thyroiditis in the majority of cases, two-thirds of whom have circulating thyroid auto-antibodies (Chapter 8).

Table 11 shows the mean age at diagnosis of diabetes in hyperthyroid and hypothyroid diabetics in relation to diabetic treatment. A distinction was again made between "primary" and "secondary" insulin-dependent diabetics. The statistical analysis was carried out using regression techniques employing the GLIM computer package.

Results

Figure 3 shows the age at diagnosis of diabetes, hyperthyroidism and hypothyroidism in the patients studied, in relation to their sex distribution. The mean ages at diagnosis of diabetes and thyroid dysfunction in hyperthyroid diabetics (46 ± 2 and 42 ± 1 years) were younger ($p < 0.001$ and $p < 0.001$) than those of hypothyroid diabetics (52 ± 2 and 58 ± 1 years). There were no significant differences between the sexes in respect of age at diagnosis of these conditions.

Seventy-six (65%) hyperthyroid and 50 (51%) hypothyroid diabetics required insulin treatment. Twenty-eight (68%) hyperthyroid and 24 (50%) hypothyroid non-insulin-dependent diabetics were treated with a sulphonylurea, the remaining non-insulin-dependent diabetics being

treated by diet with or without a biguanide. Table 11 shows that within the diabetic treatment groups of hyperthyroid and hypothyroid diabetics, the "primary" insulin-dependent diabetics were diagnosed as diabetic at a younger age than the "secondary" insulin-dependent diabetics combined with non-insulin-dependent diabetics ($p < 0.001$ and $p < 0.001$, respectively). The median age at diagnosis of diabetes in hyperthyroid and hypothyroid "primary" insulin-dependent diabetics was 36 years.

Within the group of hypothyroid, but not hyperthyroid diabetics, the "secondary" insulin-dependent diabetics were diagnosed as diabetic at a younger age ($p < 0.01$) than the non-insulin-dependent diabetics. In both the hyperthyroid and hypothyroid "secondary" insulin-dependent diabetics, the mean interval between diagnosis of diabetes and subsequent insulin treatment was 3 ± 3 years. The median durations of diabetes in hyperthyroid and hypothyroid non-insulin-dependent diabetics were seven years (range 1 - 25 years) and five years (range 1 - 18 years).

Information regarding the presence of ketonuria at diagnosis of diabetes was available in 82 hyperthyroid and 69 hypothyroid diabetics. Twenty-eight (34%) hyperthyroid and 22 (32%) hypothyroid diabetics exhibited ketonuria at diagnosis. When hyperthyroid and hypothyroid diabetics were considered together, there was a significant inverse correlation ($p < 0.001$) between the incidence of ketonuria and age at diagnosis of diabetes.

A striking correlation was observed between age at diagnosis of diabetes and thyroid dysfunction in hyperthyroid ($r = 0.71$, $p < 0.001$) and hypothyroid ($r = 0.65$, $p < 0.001$) diabetics. Similar correlations

were found when "primary" insulin-dependent diabetics ($r = 0.62$, $p < 0.001$; $r = 0.59$, $p < 0.001$), "secondary" insulin-dependent diabetics ($r = 0.42$, $p < 0.02$; $r = 0.77$, $p < 0.001$) and non-insulin-dependent diabetics ($r = 0.72$, $p < 0.001$; $r = 0.70$, $p < 0.001$) were considered independently. A similar correlation ($r = 0.81$, $p < 0.001$) was exhibited by hypothyroid non-insulin-dependent diabetics treated by diet with or without a biguanide. For hyperthyroid and hypothyroid diabetics, the regression lines for the three diabetic treatment categories did not differ significantly in slope, when age at onset of diabetes is compared with age at onset of thyroid disease.

Table 12 shows that with increasing age at diagnosis of diabetes the interval between diagnosis of diabetes and that of hyperthyroidism or hypothyroidism fell ($p < 0.001$ and $p < 0.001$). This interval was significantly longer ($p < 0.001$) in hypothyroid diabetics (6.7 ± 1.2 years) than in hyperthyroid diabetics (-2.4 ± 1.2 years)

Discussion

The Diabetic Department of the Royal Infirmary, Edinburgh, provides care for almost 90% of the local diabetic population (Falconer et al., 1971) whose female to male ratio is 1.3 : 1. A third of our general diabetic population is insulin treated, the majority of "primary" insulin-dependent diabetics being diagnosed before the age of 25 years, as reported elsewhere (Gamble and Taylor, 1969; Gamble, 1980a). In contrast, we now show that diabetics with associated autoimmune thyroid disease exhibit marked female preponderance with a female to male ratio 6.4 : 1. The proportion of insulin-dependent to non-insulin-dependent diabetics (1.4 : 1) is almost three times greater than that observed in

our general diabetic population (1 : 2). Moreover, the median age at diagnosis of diabetes in "primary" insulin-dependent diabetics with associated autoimmune thyroid disease is 36 years, that is, appreciably older than in the general diabetic population. Thus, diabetics with associated autoimmune thyroid disease display a number of clinical features at variance with the general diabetic population. On the other hand, both groups of diabetics exhibit an inverse relationship between age at onset and severity of diabetes at presentation. A similar relationship has previously been described (Gharib and Gastineau, 1969) in diabetics with associated Addison's disease suggesting that the putative autoimmune process responsible for beta cell destruction becomes less aggressive with increasing age. Conflicting reports regarding the prevalence of insulin-dependency in diabetics with associated autoimmune thyroid disease may be reconciled on consideration of the age at diagnosis of diabetes. Bottazzo et al (1978) selected patients on the basis of insulin-dependence, whose mean age at diagnosis of diabetes was 36 years. Other studies (Ganz and Kozak, 1974; Sugrue et al., 1980) which have included late-onset diabetics, report a prevalence of insulin-dependency to be 43 and 83% respectively. Our own findings clearly indicate that insulin-dependency becomes less prevalent with advancing age at diagnosis of diabetes in those patients having coexisting autoimmune thyroid disease.

The increased representation of female patients among diabetics with associated autoimmune thyroid disease is intriguing and remains unexplained. It has been demonstrated that the risk of development of diabetes in mice infected with encephalomyocarditis virus is increased by pre-treatment with testosterone (Boucher et al., 1975; Craighead and

Higgins, 1974; Morrow and Craighead, 1980) and male mice are particularly susceptible to the diabetogenic effects of streptozotocin. (Bonnie-Nielson et al., 1981) Similarly, the diabetogenic effects of N-nitroso compounds appear to be most marked in male subjects (Helgason and Jonasson, 1981), and the seasonal incidence of diabetes is most apparent when considering male juvenile-onset, insulin-dependent diabetics. Thus, environmentally induced diabetes may be associated with the male gender. In contrast, a pure autoimmune aetiology of diabetes may be associated with the female gender. It should, however, be noted that the incidence of diabetes in animal models of autoimmune insulinitis is not sex-related and this model is probably the best representation of pure autoimmune diabetes.

It is of interest that the sex ratio and age at diagnosis of autoimmune hyperthyroidism and hypothyroidism are very similar in the diabetic population, reported in this study, and non-diabetic population, reported by others (Tunbridge et al., 1977a; Sugrue et al., 1980).

The highly significant correlation between age at diagnosis of diabetes and of thyroid disease is similar to that described in respect of idiopathic Addison's disease and autoimmune ovarian failure (Irvine and Barnes, 1975). These observations support the view that where two autoimmune diseases are found to accompany one another in the same patient, both conditions are caused by a common and coincident pathogenesis. In the present study, we have shown that the correlation between age at diagnosis of diabetes and of thyroid disease is also found in non-insulin-dependent diabetics who may share the same aetiol-

ogy. The correlation extends to diet/biguanide treated hypothyroid non-insulin-dependent diabetics and cannot, therefore, be attributed to the anti-thyroid effect of the sulphonylureas (Hunton et al., 1965).

It must be said that although a strong relationship appears to link the timing of onset of diabetes and of thyroid disease, this relationship may not necessarily be due to a coincident pathogenesis, but merely to a coincidence of two life events. Alternatively, it seems possible that the pathological processes responsible for the development of diabetes and thyroid disease in the same patient are initiated simultaneously. The appearance of thyroid and gastric parietal cell antibodies appears to coincide with the onset of clinical type 1 diabetes (Riley et al., 1983) and animal models of type 1 diabetes develop thyroiditis and diabetes coincidentally (Sternthal et al., 1981).

In man, the order in which diabetes and hyperthyroidism or hypothyroidism become clinically evident appears to be related to the age at diagnosis of diabetes. Thus, whilst diabetes precedes thyroid disease in juvenile-onset diabetics, the order is reversed in late-onset diabetics. This observation may be explained by the rapid deterioration of carbohydrate tolerance in juvenile-onset diabetics leading to the early clinical diagnosis of diabetes and insulin-dependency. Late-onset diabetics run a more benign course and thus may have subclinical diabetes for several years prior to its diagnosis. Similarly, the extended natural history of asymptomatic autoimmune thyroiditis (Chapter 5) may explain why the diagnosis of hyperthyroidism precedes that of hypothyroidism irrespective of the age at diagnosis of diabetes.

In conclusion, the evidence derived from histological,

immunological, HLA and family studies (Chapter 10), indicate that type 1 diabetes and autoimmune thyroid disease are caused by a similar pathogenesis. The present study supports this premise and suggests that the pathological processes responsible for the development of diabetes and autoimmune thyroid disease in the same subject are initiated simultaneously.

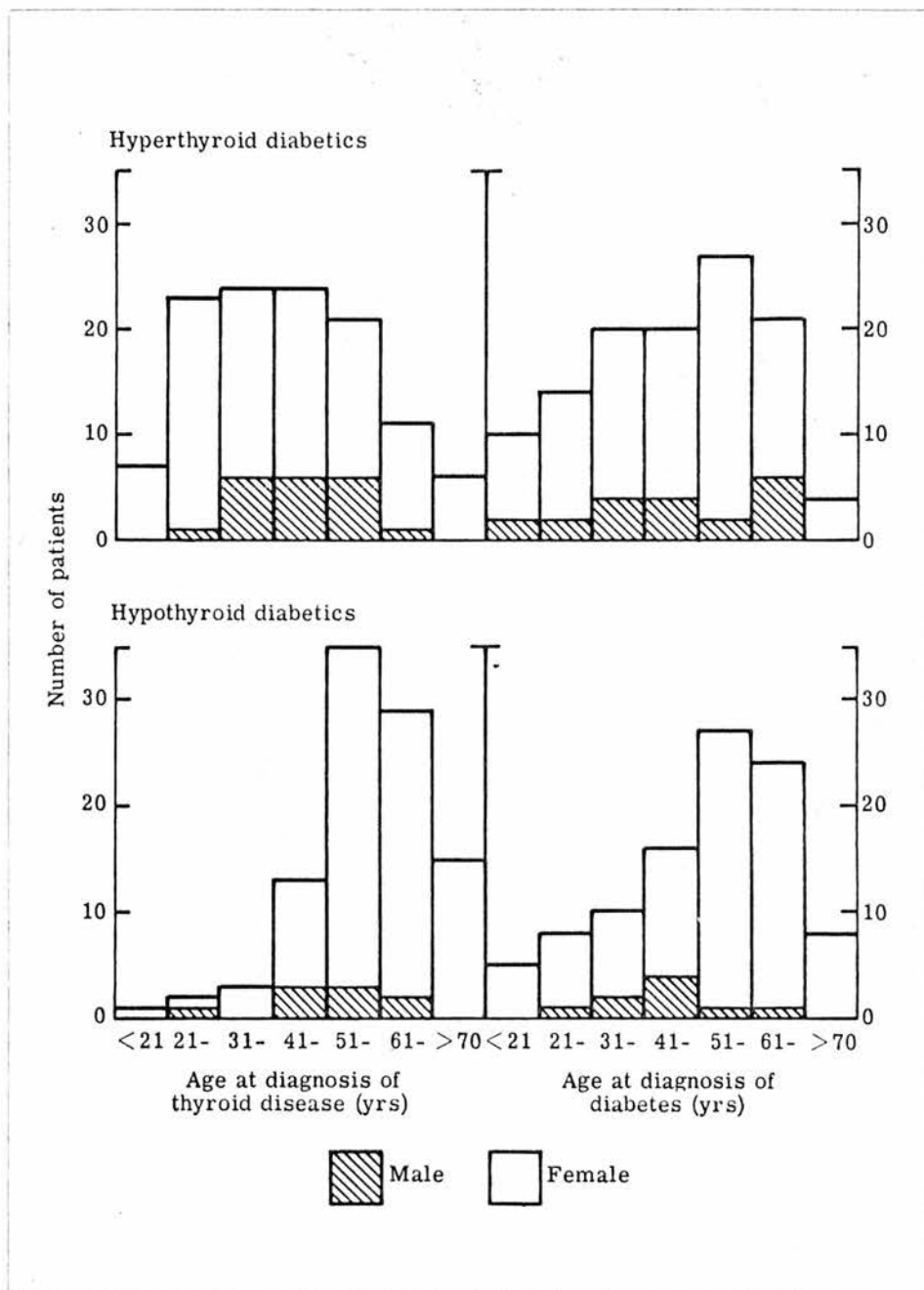


Fig. 3

Distribution of sex and age at diagnosis of diabetes, hyperthyroidism and hypothyroidism in 215 patients studied.

TABLE 11

Age at onset of diabetes in relation to diabetic treatment in hyperthyroid and hypothyroid diabetics

Thyroid disease	Diabetic treatment category	No. of patients	Age (yr.)	Significance
Hyperthyroid	"Primary" insulin-dependent diabetics	53	32 ± 14 (2-70)	p<0.001
	"Secondary" insulin-dependent diabetics	23	56 ± 10 (42-80)	
	Non-insulin-dependent diabetics	41	57 ± 10 (28-75)	NS
Hypothyroid	"Primary" insulin-dependent diabetics	30	36 ± 13 (11-65)	p<0.001
	"Secondary" insulin-dependent diabetics	20	52 ± 12 (18-71)	
	Non-insulin-dependent diabetics	48	61 ± 9 (40-79)	p<0.01

Expressed as mean ± SE († range)

TABLE 12

Interval* between diagnosis of diabetes and thyroid dysfunction according to age at diagnosis of diabetes

	Age at diagnosis of diabetes (yr)						
	<21	21-30	31-40	41-50	51-60	61-70	>70
Hyperthyroid diabetics	15(1,29)	2(-7,22)	0(-19,30)	-4(-25,7)	-6(-36,17)	-8(-36,6)	-10(-37,0)
Hypothyroid diabetics	20(-1,39)	26(-13,40)	18(0,39)	6(-22,19)	3(-8,17)	1(-10,16)	-3(-9,0)

* Presented as mean + range: where diagnosis of thyroid disease precedes that of diabetes, interval is expressed as negative

CHAPTER 7

Comparison of retinopathy in insulin-dependent diabetics with and without coexisting autoimmune thyroid disease.

The risk of developing diabetic microangiopathy is not uniformly distributed within the insulin-dependent diabetic population. A small proportion of patients seem to be subject to the early development of florid proliferative retinopathy, while other patients fail to develop significant retinopathy despite having been diabetic for forty years or more (Oakley et al., 1974). The factors governing the respective risks of the development of retinopathy in these two populations remain ill-defined. It was therefore of considerable interest when a group of workers suggested (Bottazzo et al., 1978b) that diabetics with autoimmune thyroid disease might be at particular risk of the development of retinopathy. Indirect support for this view was provided by the reported association between circulating immune complexes, commonly found in patients with autoimmune thyroid disease (Calder et al., 1974), and microangiopathy (Di Mario et al., 1980). We therefore compared the prevalence and severity of retinopathy in insulin-dependent diabetics with and without autoimmune thyroid disease to establish whether such patients are at particular risk of developing retinopathy. Since duration of diabetes has a major influence on the prevalence of retinopathy, we chose to ignore non-insulin-dependent diabetics in whom the duration of diabetes is difficult to assess.

Patients and Methods

The patients were divided into two groups according to the presence

or absence of coincident autoimmune thyroid disease. Group 1 included 60 insulin-dependent diabetics (9 males, 51 females) with Graves' disease and 105 insulin-dependent diabetics (21 males, 84 females) with primary thyroid failure. Of the patients with thyroid failure, 69 had confirmed primary hypothyroidism and 36 had normal serum thyroxine concentrations but persistently raised serum TSH concentrations. Group 2 included 164 insulin-dependent diabetics (37 males, 127 females) who had normal serum TSH concentrations and no personal history of thyroid disease. There was no significant difference between the two groups in respect of age at time of study or duration of diabetes, as shown in Table 13.

The data were submitted to χ^2 analysis.

The ocular fundi of each patient were carefully examined through dilated pupils under clinic conditions and the retinopathy status recorded 0 = no retinopathy; 1 = mild background retinopathy (small number of microaneurysms and occasional blot haemorrhage); 2 = moderate to extensive background retinopathy (large numbers of microaneurysms, blot haemorrhages, hard or soft exudates or both); 3 = new vessel formation; 4 = blindness due to vitreous haemorrhage or retinal disorganisation, or both, after haemorrhage or fibrosis.

Results

Table 13 gives the retinopathy status of patients in the two groups and shows no significant difference between them.

Discussion

No significant difference in the prevalence or severity of retinopathy was observed between insulin-dependent diabetics with and without coexisting thyroid disease. Immune complexes, as found in patients with autoimmune thyroid disease (Calder et al., 1974), most probably are not contributing to the pathogenesis of diabetic microangiopathy. Other immune complexes may well be responsible for the development of microangiopathy (Di Mario et al., 1980) but they seem to be unrelated to the coexistence of autoimmune thyroid disease.

These data do not support the hypothesis of Bottazzo et al., (1978b) that diabetes, associated with thyroid disease, is characterised by an increased risk of the development of microangiopathy. This hypothesis was based on the uncontrolled observation that retinopathy was frequently encountered within a small number of diabetics with coincident thyroid disease. This was most probably a chance finding. Moreover, the patients described had been diabetic for many years and might have therefore been expected to be at increasing risk of microangiopathy. A previous anecdotal description of apparent protection from the development of microangiopathy had been reported by Glick (1961) in a diabetic with associated cretinism. This further serves to confirm the need to carry out an appropriately controlled investigation of the risk of development of diabetic retinopathy and its putative relationship with coexisting thyroid disease. The present chapter describes such a study which refutes the suggestion that coexisting thyroid disease either protects against or increases the risk of diabetic retinopathy.

TABLE 13

Age, duration of diabetes and retinopathy status of insulin-dependent diabetics in Groups 1 and 2

Age (yr)	<20	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Group 1		3	15	24	47	49	21	6
Group 2	2	9	10	21	43	48	26	5
Duration of diabetes (yr)	5-9	10-14	15-19	20-24	25-29	30-34	35-39	>39
Group 1	30	33	33	23	22	15	6	3
Group 2	22	28	35	27	25	17	6	4
Retinopathy status	0	1	2	3	4			
Group 1	86	43	22	13	1			
Group 2	73	47	27	14	4			

CHAPTER 8

HLA antigen and serological determinants in diabetics with coexisting thyroid disease.

The clinical characteristics of diabetics with coexisting thyroid disease have previously been described (Chapter 6) and are distinct from those of the general diabetic population, but are similar to those of non-diabetics with autoimmune thyroid disease alone. Preliminary studies have suggested that thyroid/diabetics may be distinguished from subjects suffering only from diabetes or autoimmune thyroid disease on the basis of their HLA type or serological profile. Thus, in a study (Bottazzo et al., 1978a) of only 35 insulin-dependent diabetics with coexistent thyroid disease, HLA-B8 was reported to be present in 83% of thyroid/insulin-dependent diabetics - a figure almost twice that reported in the general insulin-dependent diabetic population (Cudworth and Wolf, 1982). No data have yet been published on the distribution of HLA antigens in non-insulin-dependent diabetics with thyroid disease, while the HLA antigen frequencies of hyperthyroid and hypothyroid diabetics have yet to be compared in numbers of patients sufficient to provide a meaningful answer. The frequency of islet cell, thyroid and gastric parietal cell antibodies in diabetics with associated thyroid disease is also poorly characterised. The HLA and auto-antibody status of a large group of such subjects has therefore been considered in the light of their clinical characteristics and family histories.

Patients and Methods

Patients

Two hundred and forty three diabetics were studied including 177 insulin-dependent diabetics (30 males, 147 females) and 66 non-insulin-dependent diabetics (9 males, 57 females), of whom 32 received sulphonylureas, 22 biguanides and all of whom were on an appropriate diet. Sixty-seven insulin-dependent diabetics and 27 non-insulin-dependent diabetics were hyperthyroid, and 110 insulin-dependent diabetics and 39 non-insulin-dependent diabetics were hypothyroid (including 49 insulin-dependent and 10 non-insulin-dependent diabetics having persistently elevated serum TSH concentrations, but serum thyroxine concentrations within the normal range). For hyperthyroid and hypothyroid patients, combined, the mean \pm SE ages at diagnosis of diabetes and durations of diabetes at time of study were 38 ± 1.2 years and 18 ± 0.8 years in insulin-dependent diabetics and 59 ± 1.2 years and 5 ± 0.6 years in non-insulin-dependent diabetics. Mean \pm SE ages at diagnosis of thyroid disease and durations of thyroid disease were 39 ± 1.5 years and 16 ± 1.3 years for hyperthyroid diabetics and 56 ± 1.1 years and 4 ± 0.4 years for hypothyroid diabetics, respectively.

Patients were treated with insulin if ketonuric, underweight and/or unacceptably hyperglycaemic despite treatment with combined sulphonylurea and biguanide therapy. All diabetics studied attended the Diabetic and Dietetic Outpatient Department, Royal Infirmary, Edinburgh. The clinical diagnoses of hyperthyroidism and primary hypothyroidism were confirmed biochemically by current tests of thyroid function, including estimation of plasma protein-bound iodine, effective thyroxine ratio, total T4 and TSH concentrations and by means of the TSH stimulation test. Hyperthyroidism was shown to be due to Graves' disease on the basis of clinical, histological and/or radioisotope scanning

evidence whereby patients with an overactive nodular goitre or other cause of non-Graves' hyperthyroidism were excluded.

One hundred non-diabetic controls living in Edinburgh or its environs were also studied with regard to HLA type, only.

Methods

Autoantibodies

Cytoplasmic islet cell (ICA) and complement fixing islet cell antibodies (CF-ICA) were detected as previously described (Irvine et al., 1977e; Bottazzo et al., 1980). Thyroid microsomal antibodies and gastric parietal cell antibodies were detected by the indirect immunofluorescence technique using undiluted sera. Antibodies to thyroglobulin were detected by means of the tanned cell haemagglutination technique taking a titre of ≥ 25 as significant. Serum was available from all patients.

HLA analyses

HLA typing was carried out in 143 insulin-dependent diabetics, 37 non-insulin-dependent diabetics and the controls by the standard National Institute of Health (NIH) lymphocyte cytotoxicity technique against a panel of 120 antisera defining the following locus A, B and C specificities: HLA A1, 2, 28, 3, 9 (23, 24), 10 (25, 26), 11, 19, 29, 30, 31, 32, 33. HLA B5, 7, 8, 12, (44, 45), 13, 14, 15, 16, (38, 39), 17, 18, 21, (49, 50), 22, 27, 35, 37, 40, BW4 and BW6. HLAC CW3, CW4.

Family History

A first degree family history of thyroid disease, insulin-dependent or non-insulin-dependent diabetes was ascertained by means of a postal questionnaire sent to each patient.

Statistics

Relationships between variables were tested by χ^2 analysis (with Yates' correction) or Wilcoxon rank sum tests as appropriate. Multiple logistic regression was used to test the relationship between presence of ICA or CF-ICA and other factors including duration of diabetes.

RESULTS

Autoantibodies

ICA was detected in 60 (33%) insulin-dependent diabetics and 3 (4%) non-insulin-dependent diabetics ($p < 0.001$). There was no significant difference in the prevalence of ICA when comparing hyperthyroid and hypothyroid insulin-dependent diabetics (29 vs 37%) nor between male and female insulin-dependent diabetics (17 vs 37%). Among hyperthyroid and hypothyroid insulin-dependent diabetics, combined, the prevalence of ICA declined with increasing duration of diabetes ($\chi^2 = 15.46$, $p < 0.001$), as shown in Figure 4. The same relationship was found in respect of hypothyroid, insulin-dependent diabetics ($\chi^2 = 15.74$, $p < 0.001$), but not in hyperthyroid, insulin-dependent diabetics ($\chi^2 = 0.99$, NS) when considered separately.

CF-ICA was detected in 34 (19%) insulin-dependent diabetics and 2 (3%) non-insulin-dependent diabetics ($p < 0.01$). Only one CF-ICA positive

patient was negative for ICA. There was no significant difference in the prevalence of CF-ICA when comparing hyperthyroid and hypothyroid insulin-dependent diabetics (21% vs 18%), nor between male and female insulin-dependent diabetics (10 vs 21%). Among hyperthyroid and hypothyroid insulin-dependent diabetics, combined, the prevalence of CF-ICA declined with increasing duration of diabetes ($\chi^2 = 6.12$, $p < 0.05$) as shown in Figure 4. Hypothyroid insulin-dependent diabetics considered separately showed a significant relationship between prevalence of CF-ICA and duration of diabetes ($\chi^2 = 5.52$, $p < 0.05$), but hyperthyroid insulin-dependent diabetics did not ($\chi^2 = 0.90$, NS). Age at time of study and duration of thyroid disease did not significantly influence the prevalence of ICA/CF-ICA having taken account of the relationship between prevalence of ICA/CF-ICA and diabetic treatment and duration of diabetes.

Thyroid microsomal antibodies were more common ($\chi^2 = 25.16$, $p < 0.001$) in hypothyroid (66%) than in hyperthyroid (32%) diabetics. Thyroid microsomal antibodies were more common ($\chi^2 = 7.16$, $p < 0.01$) in insulin-dependent diabetics (58%) than in non-insulin-dependent diabetics (38%). The same difference was evident when hyperthyroid and hypothyroid patients were considered separately. Thus, thyroid microsomal antibodies were more common ($\chi^2 = 4.09$, $p < 0.05$) in hypothyroid insulin-dependent diabetics (71%) than in hypothyroid non-insulin-dependent diabetics (51%) and were likewise more common in hyperthyroid insulin-dependent diabetics (31%) than in hyperthyroid non-insulin-dependent diabetics (18%), although this difference was not statistically significant.

Thyroglobulin antibodies were more common ($\chi^2 = 4.70$, $p < 0.05$) in hypothyroid (17%) than in hyperthyroid (6%) diabetics, but were not more common in insulin-dependent diabetics (12%) than in non-insulin-dependent diabetics (14%). For a given diabetic treatment and thyroid status, neither thyroid antibody showed a significant association with duration of thyroid disease or of diabetes, ICA/CF-ICA status nor with the sex nor age of the patient. The distribution of thyroid antibodies was not related to sulphonylurea treatment in hypothyroid non-insulin-dependent diabetics.

Gastric parietal cell antibodies were more common ($\chi^2 = 6.41$, $p < 0.05$) in female (37%) than male (15%) diabetics, but did not relate significantly to other autoantibody status, duration of diabetes/thyroid disease, sex, diabetic treatment or thyroid status.

HLA Analyses

Table 14 shows the distribution of those HLA antigens which are reported to be over- or under-represented in diabetics in this or previous studies. The prevalences of A1 and B8 were higher ($\chi^2 = 19.33$, $p < 0.01$ and $\chi^2 = 42.72$, $p < 0.01$) in thyroid/insulin-dependent diabetics than controls and higher ($\chi^2 = 3.99$, $p < 0.05$ and $\chi^2 = 10.73$, $p < 0.01$) than in thyroid/non-insulin-dependent diabetics. The prevalence of B7 was lower ($\chi^2 = 11.32$, $p < 0.01$) in thyroid/insulin-dependent diabetics than in controls, and lower ($\chi^2 = 5.64$, $p < 0.05$) than in thyroid/non-insulin-dependent diabetics. There were no significant differences between thyroid/non-insulin-dependent diabetics and controls in respect of HLA antigen frequencies. HLA B8 was similarly prevalent in hyperthyroid (61%) and hypothyroid (60%) diabetics (non-insulin-dependent and

insulin-dependent diabetics, combined) and in hyperthyroid (69%) and hypothyroid (65%) insulin-dependent diabetics. No HLA type was significantly related to age at onset of diabetes or of thyroid disease, nor to any of the autoantibodies tested. In particular, the relationship between prevalence of ICA/CF-ICA and duration of diabetes was not significantly different between HLA-B8 positive and negative subjects. ICA and CF-ICA were present in 34% and 18% HLA-B8 positive patients and 26% and 17% HLA-B8 negative patients.

Family histories

Family history questionnaires were completed by 94% insulin-dependent diabetics and 94% non-insulin-dependent diabetics. A first degree family history of insulin-dependent diabetes, non-insulin-dependent diabetes and thyroid disease was obtained from 20%, 9% and 19% insulin-dependent diabetics and 20%, 30% and 18% non-insulin-dependent diabetics. A positive family history of thyroid disease, insulin-dependent or non-insulin-dependent diabetes was not significantly related to autoantibody status, HLA type, age at diagnosis of diabetes or of thyroid disease.

Follow-up of thyroid/non-insulin-dependent diabetics

Since the serology of these 66 patients was determined, they have been followed up for 1 - 12 years (mean 3.2 years) in which time 5 (8%) have required insulin treatment.

Discussion

Type 1 diabetes and autoimmune thyroid disease share histological,

immunological, HLA antigen and familial characteristics as described in Chapter 1. Common to both Graves' disease (Harvald and Hauge, 1956) and type 1 diabetes (Barnett et al., 1981) is a concordance rate, in identical twins, of approximately 50% implying a strong genetically determined predisposition but also a role for an environmentally dependent aetiology. Where type 1 diabetes and autoimmune thyroid disease are found together in the same patient, both conditions commonly occur coincidentally, (Chapter 6) supporting the hypothesis of a shared underlying pathogenesis. The latter appears to become less aggressive, in respect of autoimmune beta cell destruction, with advancing age, since thyroid/diabetics developing diabetes in late middle-age are frequently non-ketonuric and non-insulin-dependent. Juvenile-onset thyroid/diabetics, on the other hand, are usually insulin requiring (Chapter 6). The prevalence of B8 in thyroid/insulin-dependent diabetics in this study (66%) is apparently greater than that described by Cudworth and Woodrow (1976) in insulin-dependent diabetics without associated autoimmune disease (51%), but not as high as previously reported (Bottazzo et al., 1978a) in a smaller series of thyroid/insulin-dependent diabetics (83%). Similarly, the prevalence of B8 in thyroid/insulin-dependent diabetics in this study is higher than has been reported in non-diabetics with Graves' disease (40%; Mather et al., 1980), atrophic hypothyroidism (53-57% ; Irvine et al., 1978b; Moens et al., 1979) or Hashimoto's hypothyroidism (26-30%; Irvine et al., 1978b; Moens et al., 1979). The present study shows that the prevalence of B8 is similar in hypothyroid and hyperthyroid insulin-dependent diabetics. It is possible that within the group of hypothyroid diabetics, further heterogeneity with respect of HLA type might

have been evident in relation to whether patients had a goitre or not, but such clinical data were not available. The over-representation of HLA-A1 in thyroid/insulin-dependent diabetics presumably reflects the previously described linkage disequilibrium between A1 and B8 (Cudworth and Woodrow, 1976). The reduced frequency of B7 in thyroid/insulin-dependent diabetics is also similar to that described (Cudworth and Woodrow, 1976) in the general insulin-dependent diabetic population. The frequency of B15 was not increased in thyroid/insulin-dependent diabetics as has been reported (Cudworth and Woodrow, 1976) in the general insulin-dependent diabetic population. It has been suggested (Rotter and Rimoin, 1978) that two distinct genotypes (B8-DW3 and B15-DW4) are associated with insulin-dependent diabetes and that only one of these (B8-DW3) is commonly found in thyroid/diabetics. Our data are in accordance with such a hypothesis.

The prevalence of ICA in the thyroid/insulin-dependent diabetics is clearly dependent upon duration of diabetes as shown in Figure 4. In insulin-dependent diabetics without associated autoimmune disease, ICA was demonstrated (Irvine et al., 1977e) in 56% and 7% of patients within one year and more than five years from diagnosis of diabetes, respectively. The equivalent figures for thyroid/insulin-dependent diabetics (77 and 30%) confirm our previous conclusion (Irvine et al., 1977e) that the persistence of ICA is a feature of diabetics having coexistent autoimmune disease. Neither the presence nor persistence of ICA is related to the sex of the patient, nor family history of thyroid disease/diabetes, nor type of thyroid disease (i.e. hyper- or hypothyroidism) and no relationship was found between HLA type and ICA. Thus, within the population of thyroid/insulin-dependent diabetics, the

persistence of ICA was not associated with HLA-B8, as has previously been described (Irvine et al., 1977e) in diabetics without overt autoimmune disease. It is possible that of the latter group, those patients demonstrating ICA persistence may subsequently have developed overt autoimmune disease. The frequency of CF-ICA has not yet been well characterised in the general diabetic population. Within thyroid/insulin-dependent diabetics, the distribution of CF-ICA mimicked that of ICA, declining in prevalence with increasing duration of diabetes, but being found in only 56% of those patients positive for ICA. Only one ICA negative patient was CF-ICA positive.

The HLA and serological characteristics of thyroid/non-insulin-dependent diabetics are clearly very different from those of thyroid/insulin-dependent diabetics. Thus, the HLA antigen distribution of thyroid/non-insulin-dependent diabetics is not significantly different from that of the control population and only the minority of thyroid/non-insulin-dependent diabetics are positive for ICA or CF-ICA. As such, it is very possible that thyroid/non-insulin-dependent diabetics are type 2 diabetics representing the chance coincidence of two relatively common disorders - autoimmune thyroid disease and type 2 diabetes. The low incidence of subsequent insulin dependency exhibited by the thyroid/ non-insulin-dependent diabetics would appear to substantiate the view that these are type 2 diabetics. On the other hand, thyroid/non-insulin-dependent diabetics tend to develop diabetes and autoimmune thyroid disease coincidentally (Chapter 6) suggesting a common underlying pathogenesis. Furthermore, the familial prevalence of insulin-dependent diabetes and autoimmune thyroid disease is very high in close relatives of thyroid/ non-insulin-dependent diabetics (Chapter

10), implying that an inherited susceptibility to autoimmunity is operative in such families. This predisposition appears not to be linked to HLA-B8, and if the underlying pathogenesis of diabetes and thyroid disease is uniform in such patients, and is immunologically mediated, it is characterised by the relative absence of autoantibodies. Thus, thyroid/non-insulin-dependent diabetics have significantly lower frequencies of ICA/CF-ICA and thyroid microsomal antibodies than thyroid/insulin-dependent diabetics. The low frequency of thyroid microsomal antibodies in thyroid/non-insulin-dependent diabetics cannot be attributed to differences in age, duration of thyroid disease or sex between the two groups of diabetics, and cannot be related to the reported antithyroid effects of sulphonylurea treatment (Hunton et al., 1965). The possibility, therefore, remains that thyroid/non-insulin-dependent diabetics have an underlying diabetic pathogenesis of lesser virulence, which is characterised by a less frequent or less sustained humoral response than is found in thyroid/insulin-dependent diabetics, leading to incomplete beta cell destruction. Other examples of autoimmune diseases having varying degrees of severity and clinical expression include Graves' disease whose course, after antithyroid drug therapy, depends on HLA and serological determinants (Irvine et al., 1977b; McGregor et al., 1980). Similarly, the natural history of autoimmune thyroiditis is non-uniform and is related to serological factors as described in Chapter 5.

Bottazzo et al (1978a) have suggested that thyroid microsomal antibodies are found in a higher proportion of hyperthyroid/insulin-dependent diabetics (93%) than in uncomplicated hyperthyroid patients (70%). Our findings do not confirm this difference with equivalent

frequencies of 31% in hyperthyroid/insulin-dependent diabetics and 61% in uncomplicated hyperthyroidism (Irvine et al., 1977d). Gastric parietal cell antibodies were found in thyroid/diabetics with a frequency similar to that previously reported (Irvine et al., 1970) in the general diabetic population, in whom the sex difference was again noted.

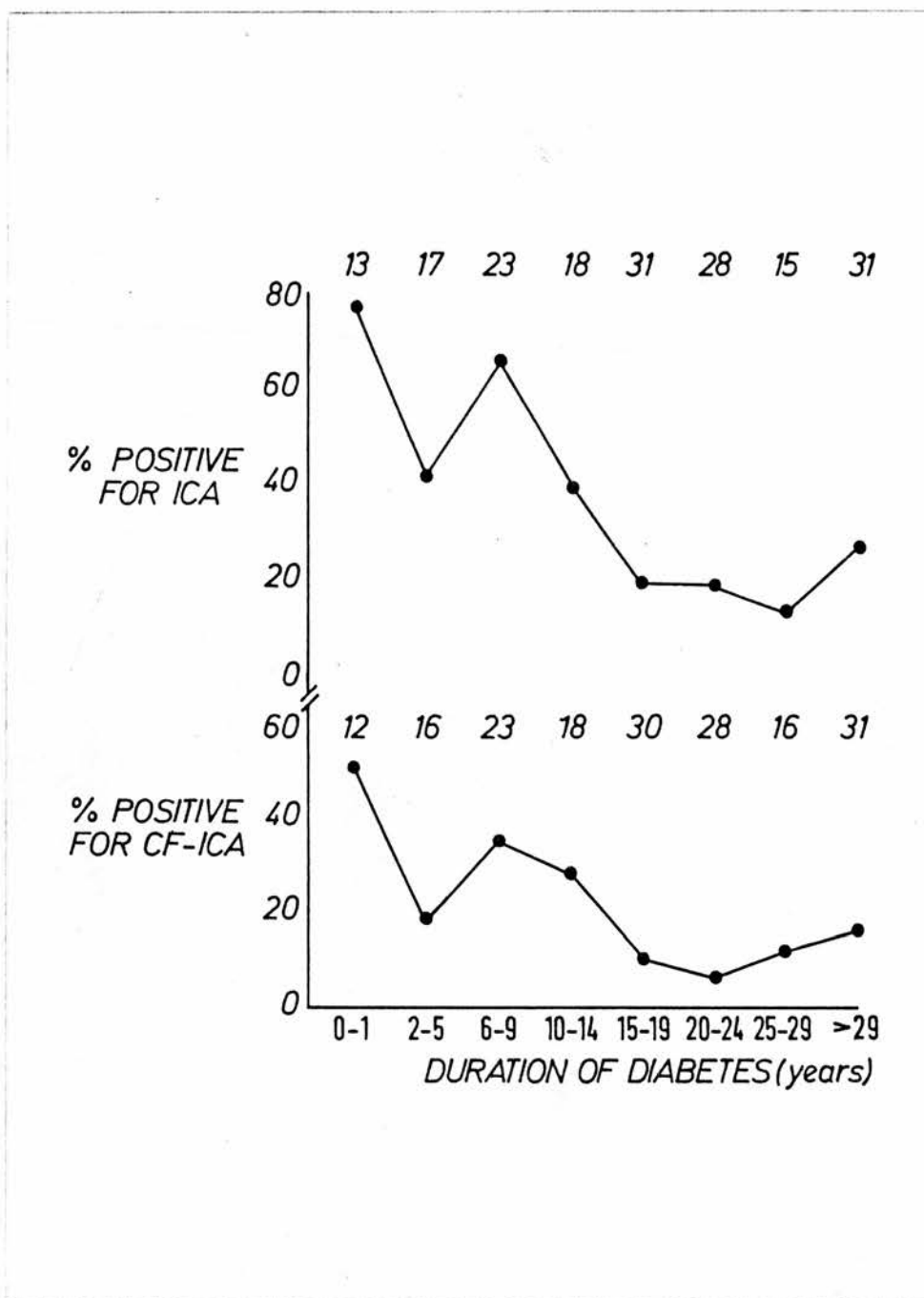


Fig. 4

Prevalences of ICA and CF-ICA, according to duration of diabetes, in insulin-dependent diabetics with coexisting autoimmune thyroid disease.

TABLE 14

HLA antigen frequencies (%) in study groups

HLA antigen	Insulin-dependent diabetics		Non-insulin-dependent diabetics		Controls n=100
	Hyperthyroid n=59	Hypothyroid n=89	Hyperthyroid n=19	Hypothyroid n=28	
A1	59	52	35	35	31
B7	14	14	35	30	26
B8	69	65	37	36	23
B15	14	14	18	5	10

1
9
3
1

PART 4

CHAPTER 9

Seasonal incidence of type 1 diabetes in diabetics with and without autoimmune thyroid disease.

In 1926, Adams demonstrated "a seasonal variation in the onset of acute diabetes" irrespective of sex and age at onset. He postulated that this phenomenon might result from the seasonal variation in physical activity and susceptibility to non-specific infection of an individual genetically predisposed to diabetes. A seasonal variation in the incidence of insulin-dependent diabetes has since been confirmed in patients presenting under the age of 20 years (Gamble and Taylor, 1969; Christau et al., 1977; Bloom et al., 1975; Fishbein et al., 1982). Where an attempt has been made (Gamble and Taylor, 1969) to show a seasonal variation in the incidence of insulin-dependent diabetes presenting over the age of 20 years, the evidence is less convincing although the decline in incidence during the early summer months remains evident when all patients below the age of 30 years are considered together (Christau et al., 1977), or when all patients are considered irrespective of age at onset (Adams, 1926). If the seasonal variation in incidence provides a clue to the aetiology of insulin-dependent diabetes, it is necessary to establish which patients exhibit this phenomenon. We have, therefore, examined the effects of sex and age at onset on the pattern of seasonal incidence of insulin-dependent diabetes, and have specifically considered insulin-dependent diabetics with coexisting autoimmune thyroid disease to see whether they also exhibit a seasonal variation in incidence.

Patients and Methods

All patients with newly diagnosed diabetes who had been referred to the Diabetic and Dietetic Department, Royal Infirmary, Edinburgh from the city or its environs between 1964 and 1977 (inclusive) were considered. Patients were regarded as insulin-dependent according to their age at presentation, if under weight and/or ketonuric and only those treated with insulin from diagnosis and for at least 6 months thereafter were included. Patients with secondary diabetes, due to pancreatic disease, endocrine disturbance or other causes were excluded. The study did not include patients aged less than 10 years and many others aged 10 to 13 years who had been referred initially to a paediatric clinic. The majority (i.e. almost 90%) of diabetics in Edinburgh attend the Diabetic Department at the Royal Infirmary (Falconer et al., 1971) and will have, therefore, been considered in our study. These included patients with and without autoimmune thyroid disease, no attempt having been made to segregate the two groups.

Five hundred and two patients (297 males, 205 females) were included and analysed for the year and month of diagnosis and of symptomatic onset of diabetes. The duration of diabetic symptoms before diagnosis had been obtained from all but nine patients. For the purpose of further analyses, patients were sub-divided into three groups according to age at onset (10-19, 20-29 and more than 29 years).

Statistical analysis of the seasonal variation in incidence was made by a test due to David and Newell (1965). The duration of symptoms of diabetics of differing sex and age at onset were compared using Cox's test for trend (Cox, 1970).

Results

The age at diagnosis and sex distribution of the 502 diabetics studied are shown in Figure 5. The mean annual yield of newly-diagnosed insulin-dependent diabetics was 14.8 (range 8-22) for patients aged 10-19, 10.9 (range 5 - 19) for those aged 20-29 and 10.0 (range 6 - 15) for those aged more than 29 years.

Figure 6A compares the seasonal variation in incidence of diagnosis of diabetes in the three age groups where patients of both sexes were combined. In patients aged 10 - 19 years at diagnosis, the incidence of diagnosis was significantly greater during the winter months than during the summer months ($p < 0.05$). Patients aged 20-29 and more than 29 years failed to show significant seasonal variation in the incidence of diagnosis. When males and females aged 10-19 and more than 19 years (combining the patients aged 20-29 and more than 29 years at diagnosis) were considered separately (Table 15), only the males aged 10 - 19 years showed a significant seasonal variation in incidence of diagnosis ($p < 0.05$).

Figure 7 compares the duration of diabetic symptoms experienced by diabetics of different sex and age groups. When the sexes were considered together, the duration of symptoms of diabetics aged 10-19 years was significantly shorter than that of diabetics aged 20-29 years ($p < 0.02$) and diabetics aged more than 29 years ($p < 0.0001$). Women within each age group had a longer duration of symptoms than men but the difference was only statistically significant ($p < 0.05$) in patients aged 20-29 years at diagnosis.

Figure 6B shows a seasonal variation in symptomatic onset of diabetes in 421 patients according to age at diagnosis, having excluded 72 patients whose symptoms had been present for more than three months prior to diagnosis and nine patients whose duration of symptoms was unknown. In patients of both sexes combined, aged 10-19 years at diagnosis, the incidence of symptomatic onset was again commoner during the winter months than during the summer months ($p < 0.01$). Patients of both sexes combined aged 20-29 years and more than 29 years at diagnosis failed to show a seasonal variation in symptomatic onset. When males and females aged 10-19 and more than 19 years (combining the patients aged 20-29 and more than 29 years at diagnosis) were considered separately (Table 15), only the males aged 10-19 years showed a significant seasonal variation in incidence of symptomatic onset ($p < 0.05$).

Discussion

Allowing for the fact that some younger teenagers and patients aged less than 10 years are referred to paediatric clinics, the distribution of age and sex at presentation of our patients is in accordance with reports from other centres (Gamble and Taylor, 1969; Cudworth et al., 1977), showing a slight male preponderance until middle age with female preponderance in the older age groups. As with other studies, we observed a seasonal variation in incidence of insulin-dependent diabetes presenting between the ages 10-19 years - the incidence being higher between the months of September and April inclusive. Previous investigation of patients presenting below the age of 6 years has revealed a lack of seasonal variation in incidence (Bloom et al., 1975; McMillan et al., 1977; Fishbein et al., 1982), while we have shown the same to be

true of patients aged over 19 years at presentation.

A significant seasonal variation in incidence of insulin-dependent diabetes might only be evident when duration of symptoms prior to diagnosis is taken into account and those patients having a particularly lengthy symptomatic onset excluded. Thus, the seasonal variation in symptomatic onset is more marked than the seasonal variation in diagnosis, when considering patients aged 10-19 years at presentation. Once again, patients aged more than 19 years failed to show seasonal variation in symptomatic onset, although it is evident that the duration of symptoms prior to diagnosis increases with increasing age at onset of diabetes. Such a prolonged interval between onset and diagnoses of diabetes may obscure the seasonal variation in incidence as shown by the younger patients whose duration of symptoms is relatively short.

A further important observation is that females do not demonstrate a seasonal variation in diagnosis or symptomatic onset whichever age category is considered, whereas males aged 10-19 years exhibit a significant seasonal variation in incidence. Duration of symptoms is consistently longer in females than male patients, and this may help to obscure a true seasonal variation in incidence. Since the number of female patients studied was relatively small, it might be argued that a significant variation in incidence would emerge on examining a larger population. However, Fishbein et al (1982) have recently reported females to lack seasonal variation in incidence when examining 901 children aged less than 20 years at diagnosis. Males aged 5-14 years again showed a significant seasonal variation in incidence.

If a variable seasonal incidence is regarded as supportive evidence

of an environmentally induced aetiology, then male teenagers appear to be particularly susceptible to this pathogenesis of insulin-dependent diabetes. Lack of seasonal variation in incidence may imply an alternative pathogenesis unrelated to environmental influences which may be operative for female insulin-dependent diabetics. Indirect support for this view derives from the observation that male animals are particularly vulnerable to pancreatic islet injury induced by streptozotocin (Bonnievie-Nielsen et al., 1981), viral infections (Boucher et al., 1975; Craighead and Higgins, 1974; Morrow and Craighead, 1980) and other compounds as found in smoked-cure mutton, (Helgason et al., 1982).

Since diabetics with associated autoimmune thyroid disease are usually female and commonly late-onset, they might be expected not to show a seasonal variation in incidence. In order to confirm this, a study was undertaken of the month of diagnosis and of symptomatic onset in a group of insulin-dependent diabetics with associated thyroid disease.

Patients and Methods

Those diabetics having coexisting autoimmune thyroid disease, (n = 83) who were treated with insulin from diagnosis ("primary" insulin-dependent diabetics) and whose clinical features were described in Chapter 5, were studied together with 33 patients (13 males, 20 females) who were found to have persistently elevated serum TSH concentrations as evidence of primary thyroid failure, the majority of whom had thyroid autoantibodies. These patients were also treated with insulin from diagnosis. The patients thus included 53 hyperthyroid patients whose mean \pm SE age at diagnosis of diabetes was 32.4 ± 13.9 years, and 63 patients with primary thyroid failure, whose mean age at diagnosis of

diabetes was 38.2 ± 14.3 years.

Analysis of the seasonal variation in onset of diabetes was made by the test of David and Newell (1965).

Results

Figure 8 compares the distribution of duration of diabetic symptoms experienced by all "primary" insulin-dependent diabetics (including patients with and without autoimmune thyroid disease) referred to the Diabetic and Dietetic Department, Royal Infirmary, Edinburgh between 1964 and 1977 with that of "primary" insulin-dependent diabetics with coexisting autoimmune thyroid disease referred to the same clinic. Only patients treated with insulin from the outset were considered. It may be seen that the two groups of patients had similar durations of symptoms.

Figure 9A shows the seasonal incidence of diagnosis of diabetes in "primary" insulin-dependent diabetics with coexisting thyroid disease. Figure 9B shows the seasonal incidence of symptomatic onset of diabetes in the same patients, having excluded those whose duration of symptoms exceeded three months or had not been recorded. No significant seasonal variation in incidence of diagnosis nor symptomatic onset of diabetes was observed.

Discussion

The duration of diabetic symptoms at diagnosis is not significantly longer in insulin-dependent diabetics with coexisting autoimmune thyroid disease than in the general insulin-dependent diabetic population. Hav-

ing allowed for the interval between the onset of symptoms and diagnosis of diabetes, diabetics with associated thyroid disease do not exhibit a seasonal variation in incidence, nor peaks in age at diagnosis of diabetes as shown by Figure 5. There is therefore no epidemiological evidence to incriminate an environmentally determined aetiology in the pathogenesis of diabetes when associated with autoimmune thyroid disease, as provided by the clear seasonal variation in incidence of male, teenager insulin-dependent diabetics. Analogous heterogeneity is found amongst the pathologies of the thyroid gland such that patients may suffer from viral subacute thyroiditis on the one hand and a variety of autoimmune syndromes on the other hand. It seems likely that similar heterogeneity may be found within the insulin-dependent diabetic population. Thus, a proportion of insulin-dependent diabetics may develop insulinitis as a result of a viral infection, the evidence for which is outlined in Chapter 1. Such patients often develop diabetes in childhood, are predominantly male, exhibit a seasonal variation in incidence and show a higher prevalence of HLA-B15 than older onset insulin-dependent diabetics. Conversely, diabetics with associated thyroid disease commonly develop diabetes in middle age, are predominantly female, do not show any seasonal variation in incidence and show an increased prevalence of HLA-B8. The aetiology of diabetes in such patients may be determined by a true autoimmune pathogenesis unrelated to environmental factors.



Fig. 5

Age at diagnosis of diabetes and sex distribution of patients studied.

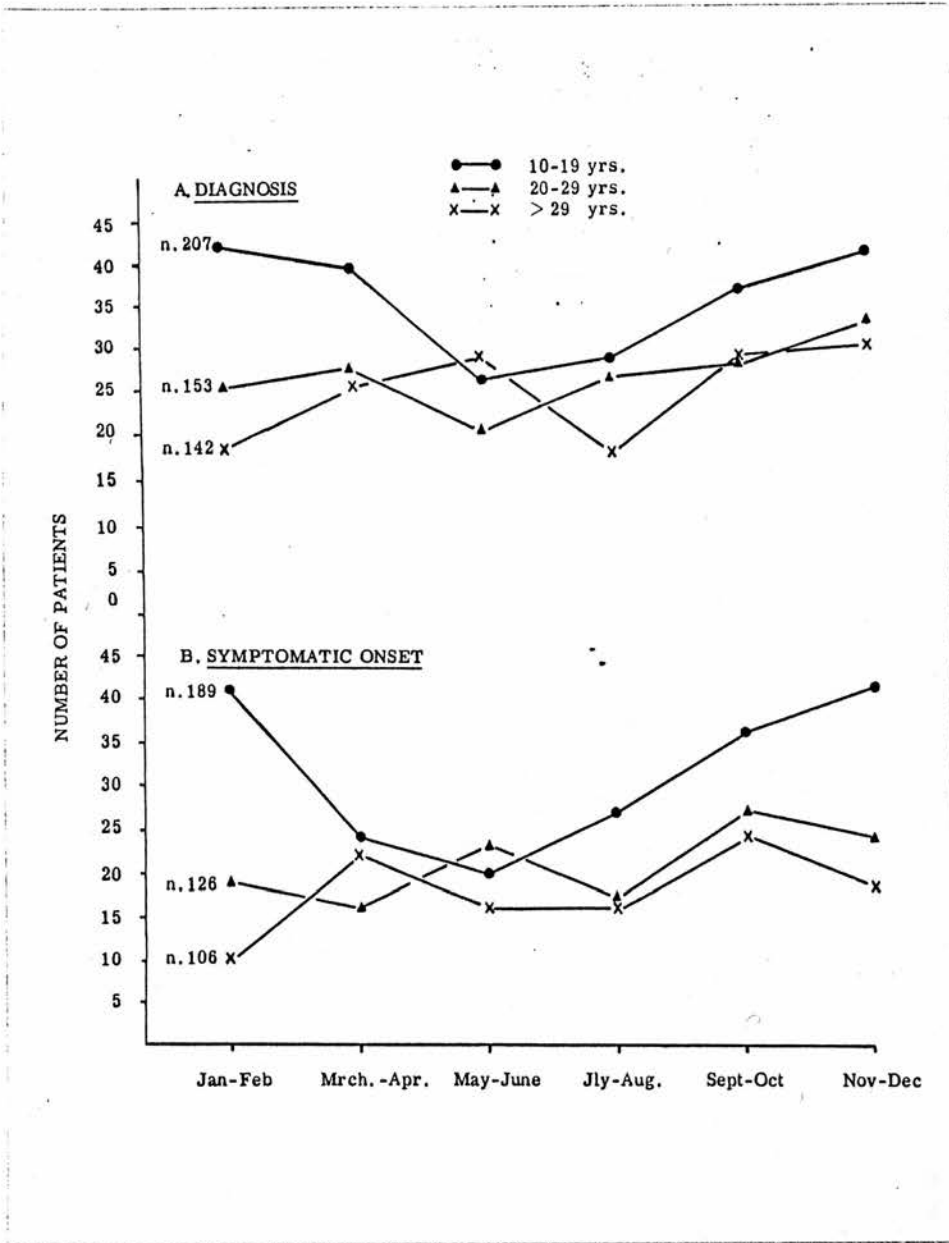


Fig. 6

Pattern of seasonal incidence of diagnosis (A) and of symptomatic onset (B), of insulin-dependent diabetes according to age at onset.

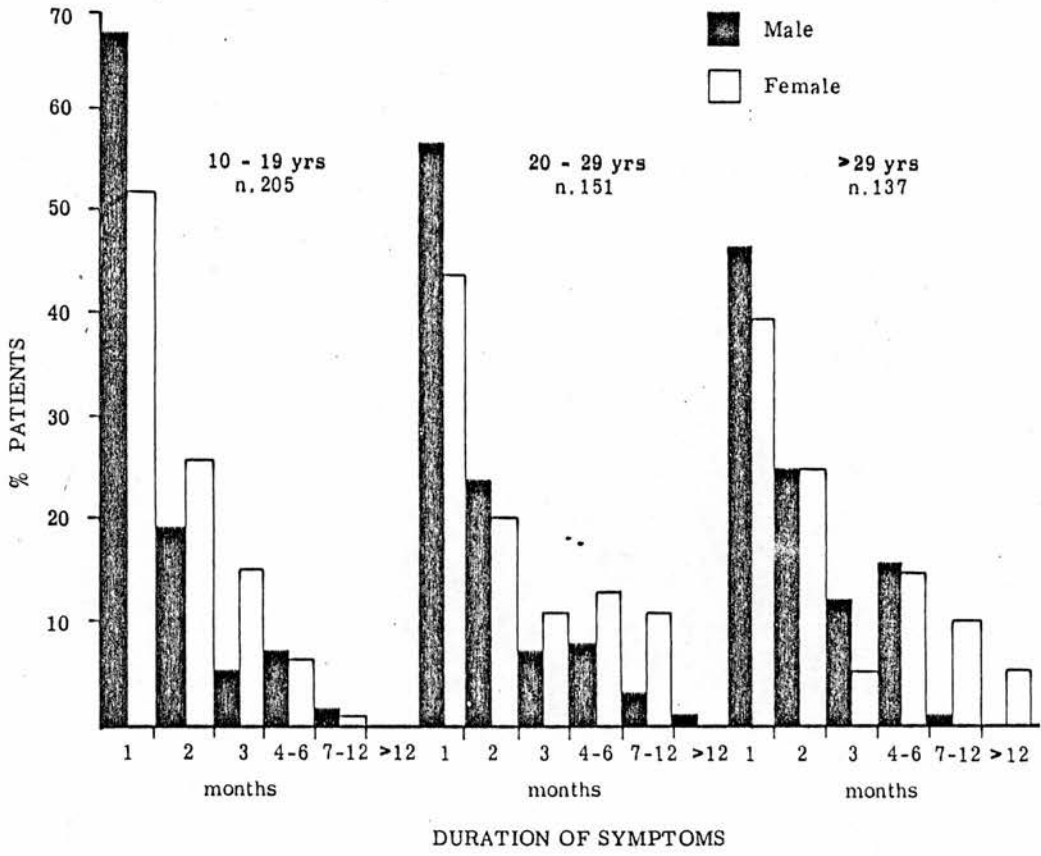


Fig. 7

Distribution of duration of symptoms according to age at onset and sex.

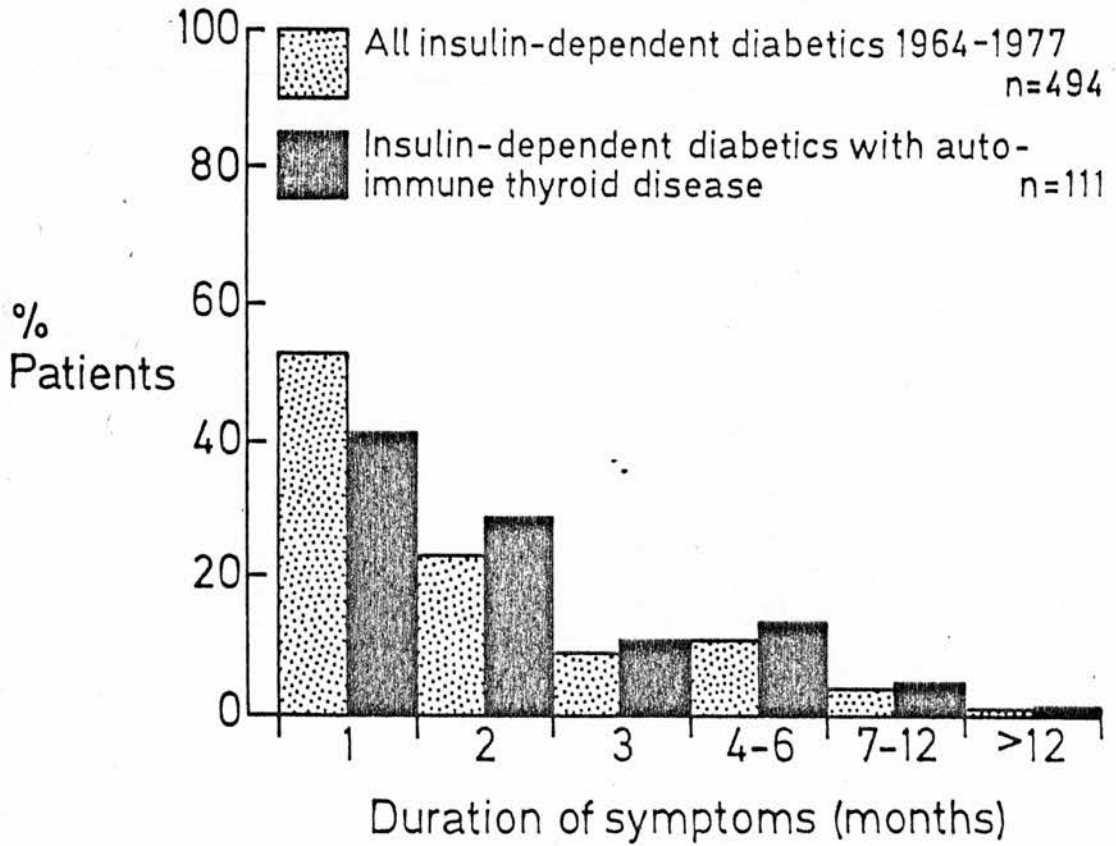


Fig. 8

Comparison of duration of symptoms of insulin-dependent diabetics with coexisting autoimmune thyroid disease with that of all insulin-dependent diabetics presenting between 1964 and 1977.

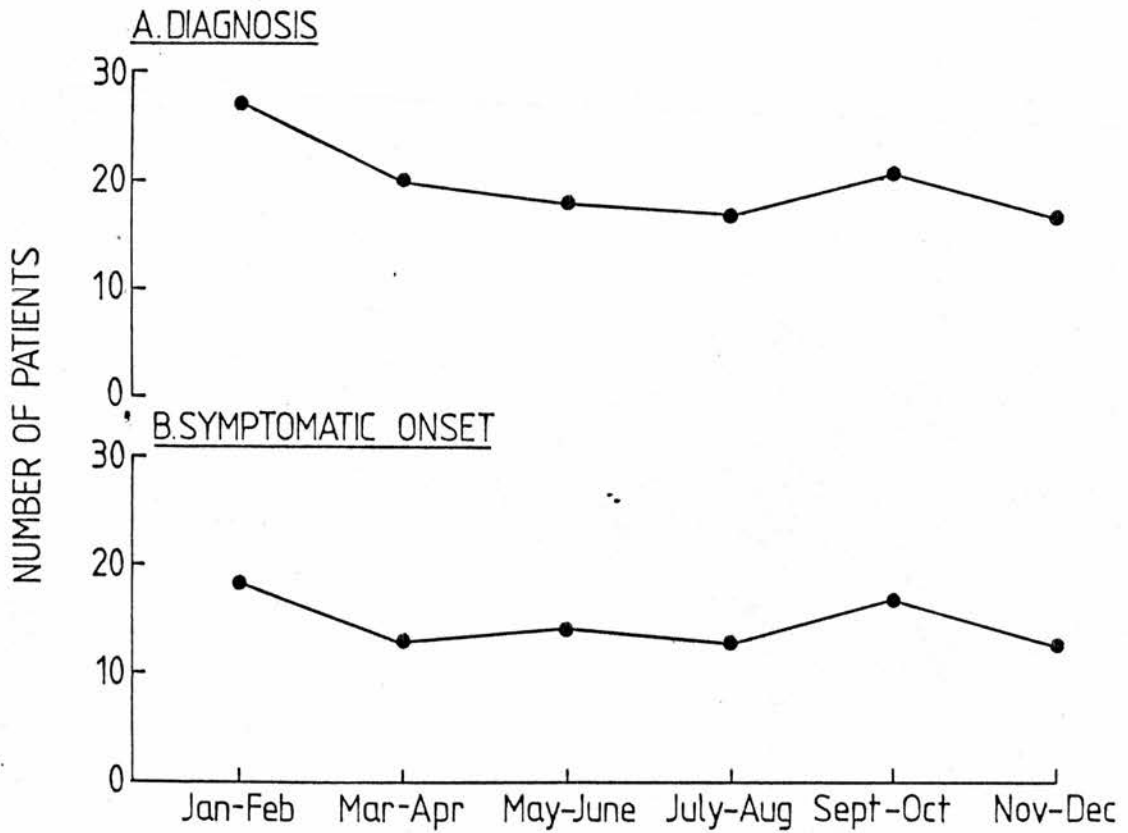


Fig. 9

Lack of seasonal incidence of diagnosis (A), and of symptomatic onset (B), of insulin-dependent diabetes in patients with coexisting autoimmune thyroid disease.

TABLE 15

Diagnosis and symptomatic onset of male and female insulin-dependent diabetics according to age at onset and time of year

Diagnosis	Age (yr.)	Sex	Jan-	March-	May-	July-	Sept-	Nov-
			Feb	April	June	Aug	Oct	Dec
Diagnosis	10-19	M	21	22	13	11	22	29
		F	21	17	12	16	13	10
	>19	M	25	33	32	27	29	33
		F	18	19	15	14	24	26
Symptomatic onset	10-19	M	20	15	8	16	22	27
		F	21	9	12	11	14	14
	>19	M	18	25	25	23	30	28
		F	9	15	13	11	21	14

CHAPTER 10

Family studies in diabetics with and without autoimmune thyroid disease.

In the preceding chapters the frequency with which insulin-dependent diabetes and autoimmune thyroid disease coexist in the same patient was described, and it was emphasised how such patients may be distinguished from other diabetics without associated thyroid disease on the basis of clinical, HLA antigen and serological determinants. Evidence was presented for an environmentally dependent aetiology in the pathogenesis of some forms of insulin-dependent diabetes and the lack of such evidence in diabetics having coexisting thyroid disease. Since familial aggregation is a striking feature of other organ-specific autoimmune diseases, the distribution of insulin-dependent diabetes in close relatives of autoimmune diabetics (i.e. those with coexistent autoimmune thyroid disease) might also be characteristic and distinct from that of non-autoimmune diabetics (i.e. those without coexistent autoimmune thyroid disease). In order to test this hypothesis, the patterns of inheritance of thyroid disease, insulin-dependent and non-insulin-dependent diabetes in first degree relatives of diabetics with and without coexisting autoimmune thyroid disease were compared.

PATIENTS AND METHODS

Diabetics with thyroid disease

Two hundred and thirty three unrelated diabetics (38 males, 195 females) were included who had clinically evident hyperthyroidism (n=86) or primary hypothyroidism (n=115). The clinical diagnoses of hyperthyroidism and primary hypothyroidism were confirmed biochemically by

current tests of thyroid function, including estimation of plasma protein-bound iodine, effective thyroxine ratio, total thyroxine and thyrotrophin (TSH) concentrations and by means of the TSH stimulation test. Hyperthyroidism was shown to be due to Graves' disease on the basis of clinical, histological and/or radioisotope scanning evidence whereby patients with an overactive nodular goitre or other cause of non-Graves' hyperthyroidism were excluded. Thirty two patients with clinically unrecognised impaired thyroid reserve, having persistently elevated plasma TSH concentrations ($>5.7\mu\text{u/l.}$), were also included. Since the majority of diabetics with primary thyroid failure have circulating thyroid auto-antibodies, the aetiology of hypothyroidism may be considered to be autoimmune thyroiditis.

Diabetics without thyroid disease

Four hundred and ten unrelated diabetics (159 males, 251 females) were included who had no previous history of thyroid disease and who had been shown to have a normal plasma TSH concentration. These were selected on the basis that they should be of similar age, diabetic duration and diabetic treatment as the diabetics with thyroid disease.

All diabetics attended the Diabetic and Dietetic Out-Patient Department at the Royal Infirmary, Edinburgh.

Controls

Five hundred and eight healthy volunteers (277 males, 231 females) were included, who had no medical history of diabetes or thyroid disease. They comprised 98 males, aged 40-59 years, who were attending the Heart Disease Prevention Clinic, 111 females, aged 40-59 years,

who were attending the Edinburgh Breast Screening Clinic and 299 blood donors (179 males, 120 females) who were selected on the basis that they should be of similar age to the diabetic groups.

A postal questionnaire was sent to all participants asking the number of siblings and children in each participant's family and whether any first degree relatives were known to have had an overactive or underactive thyroid gland, insulin-dependent or non-insulin-dependent diabetes. Age at onset of diabetes in affected relatives was also asked. A brief summary of the respective symptoms and likely treatments of each condition was provided. In order to validate the reported status of diabetic relatives of diabetic propositi, an attempt was made to examine the hospital records of all such diabetic relatives attending the Diabetic and Dietetic Outpatient Department, Royal Infirmary, Edinburgh. Records for 54 (51%) of the 105 diabetic relatives of diabetics with thyroid disease and 52 (37%) of the 140 diabetic relatives of diabetics without thyroid disease were available. All such patients were confirmed to be diabetic and their treatment had been correctly reported by postal questionnaire in every case.

Statistical analysis

Statistical comparisons of the prevalence of affected relatives among the various groups of propositi were complicated by the fact that the propositi had variable numbers of siblings and children at risk. Thus the denominators for the proportions of affected siblings and children given in Tables 7, 9 and 22 are the total numbers of the particular type of relative in each group, and not the numbers of propositi in the groups. A simple comparison of proportions between two groups by a 2x2

and non-autoimmune groups of propoiti each showed male preponderance in children and female preponderance in siblings and parents. When all insulin-dependent relatives were considered together, the male to female ratio in children (5.66:1) exceeded that (0.68:1) of siblings and parents combined ($\chi^2 = 12.25$, $p < 0.001$). This difference might be due to males developing insulin-dependent diabetes at a younger age than females. However, mean age at onset of insulin-dependent diabetes in affected relatives was similar in males and females when each generation was considered separately (Table 20). The sex ratio (M:F) of insulin-dependent diabetic propoiti with insulin-dependent diabetic sons (4:9) was not significantly different from that (2:1) of those with insulin-dependent diabetic daughters.

The sex of the propoitus did not significantly influence the prevalence of insulin-dependent diabetes in first degree relatives, whichever generation of relative or study group was considered.

Table 21 compares the observed and expected prevalences of insulin-dependent diabetes in parents of the five study groups, based on the observed prevalence in siblings. The observed exceeded the expected prevalence when considering parents of controls ($\chi^2 = 5.22$, $p < 0.05$) or those of all subjects without a personal/family history of thyroid disease ($\chi^2 = 9.76$, $p < 0.001$). In contrast, the expected exceeded the observed prevalence, albeit not significantly so ($\chi^2 = 2.24$), when considering parents of insulin-dependent and non-insulin-dependent diabetics with a personal/family history of thyroid disease. Comparison of the ratios of observed to expected prevalence of insulin-dependent diabetes in parents of subjects with and without a

difference was observed.

2. Family history of insulin-dependent diabetes.

Of 266 diabetics with a personal history (n=233) or only a first degree family history (n=33) of thyroid disease, 179 were insulin-dependent and 87 were non-insulin-dependent. Diabetics with neither a personal nor first degree family history of thyroid disease included 245 insulin-dependent and 111 non-insulin-dependent patients.

Table 19 shows the distribution of insulin-dependent diabetes in relatives of the five study groups. Insulin-dependent diabetes was less common ($\chi^2 = 7.52$, $p < 0.01$) in mothers of controls than mothers of insulin-dependent diabetics without a personal/family history of thyroid disease. With this exception, the prevalence of insulin-dependent diabetes was similar in parents and children of the five study groups.

The prevalence of insulin-dependent diabetes in siblings of insulin-dependent diabetics with a personal/family history of thyroid disease was greater ($\chi^2 = 7.71$, $p < 0.01$) than that of insulin-dependent diabetics without a personal/family history of thyroid disease which was, in turn, greater ($\chi^2 = 8.81$, $p < 0.01$) than that of controls. The prevalence of insulin-dependent diabetes in siblings of non-insulin-dependent diabetics with a personal/family history of thyroid disease was greater ($\chi^2 = 6.53$, $p < 0.05$) than that of non-insulin-dependent diabetics without a personal/family history of thyroid disease and greater ($\chi^2 = 7.95$, $p < 0.01$) than that of controls.

Table 20 compares the sex ratios of insulin dependent diabetic relatives of autoimmune and non-autoimmune propoiti. The autoimmune

thyroid disease, 17 (12%) were male, with an overall female to male ratio of 7.2:1. On the other hand, the prevalences of thyroid disease in relatives of male and female probands were similar.

The reported prevalences of hyperthyroidism and hypothyroidism in relatives of the five study groups were similar and are reflected by the overall figures for thyroid disease shown in Table 17. Of 139 affected relatives, 90 (65%) were hyperthyroid and 49 (35%) were hypothyroid. The reported prevalences of hyperthyroidism and hypothyroidism amongst first degree relatives of hyperthyroid probands (18% and 6%) were not significantly different from those (15% and 12%) amongst relatives of hypothyroid probands.

The question was then considered as to whether thyroid disease was found in similar prevalence in successive generations of relatives of the study groups. Given the observed prevalence of thyroid disease in siblings of probands, it is possible to estimate the expected prevalence in parents, although it must be remembered that this is a conservative estimate since parents will be older than siblings and will therefore be at greater risk of having developed thyroid disease. Table 18 shows a comparison between the observed and expected prevalences of thyroid disease in parents of subjects in the five study groups. In contrast to the other groups, the observed prevalence was lower than expected in respect of parents of insulin-dependent diabetics with thyroid disease ($\chi^2 = 4.36, p < 0.05$) and non-insulin-dependent diabetics with thyroid disease (N.S.). However, when parents of all subjects with and without thyroid disease were compared in respect of ratio of observed to expected prevalence of thyroid disease, no significant

145 were insulin-dependent and 82 were non-insulin-dependent. Diabetics without thyroid disease included 279 insulin-dependent and 116 non-insulin-dependent patients. The characteristics of all participants (n = 1100) in the 5 study groups are shown in Table 16.

1. Family history of thyroid disease

Table 17 shows the distribution of thyroid disease in relatives of the five study groups. Thyroid disease was of similar prevalence in parents and children of the five groups.

The prevalence of thyroid disease in siblings of insulin-dependent and non-insulin-dependent diabetics with coexisting thyroid disease were similar and greater ($\chi^2 = 6.80$, $p < 0.01$ and $\chi^2 = 3.95$, $p < 0.05$) than that of insulin-dependent diabetics without thyroid disease. The prevalence of thyroid disease in siblings of insulin-dependent diabetics without coexisting thyroid disease was greater ($\chi^2 = 4.83$, $p < 0.05$ and $\chi^2 = 6.00$, $p < 0.05$) than those of non-insulin-dependent diabetics without thyroid disease and controls, which were similar.

Twenty-nine (20%) insulin-dependent diabetics with thyroid disease, 21 (26%) non-insulin-dependent diabetics with thyroid disease, 34 (12%) insulin-dependent diabetics without thyroid disease, 5 (4%) non-insulin-dependent diabetics without thyroid disease and 28 (6%) controls had a first-degree family history of thyroid disease.

Consistent female preponderance amongst relatives having thyroid disease was noted, irrespective of propositus group, generation of relative or type of thyroid disease (hyperthyroid or hypothyroid) affecting the propositus or affecting the relative. Of 139 relatives with

contingency table analysis is therefore invalid, since some of the individuals making up the totals belong to the same family and so are not independent. In particular, a situation in which the chance of an individual being affected by (say) thyroid disease was strongly dependent on familial factors but was independent of diabetic status could result in a spurious significance in a comparison of prevalence of thyroid disease in the relatives of two different groups of propositi classified by diabetic status. It was therefore necessary to analyse the prevalence data by comparing the proportions of propositi reporting at least one affected relative. Clearly the chance of a propositus doing so will depend both on his or her own age and on the number of that type of relative he or she has, as well as (perhaps) on his or her own disease status. Multiple logistic regression analysis was therefore used to test whether the different groups of propositi differed in their probabilities of reporting affected relatives once the effects of age and family size were accounted for. The age and family size effects were in fact quite significant in many of the analyses, suggesting that the use of this method was indeed necessary. For parental prevalence, only the age effect was included, since the number of parents per propositus is of course constant. The method results in an approximate chi-squared statistic with one degree of freedom when two groups are compared.

The prevalence of affected parents and siblings was also compared within groups (Tables 18, 21 and 23) by a method involving the calculation of the expected number of affected parents under the hypothesis that parents and siblings are equally likely to be reported as affected. The method is described here for a comparison of mothers with sisters.

For a propositus with n sisters and r affected female relatives in the parental and sibling generations, the probability of the mother being affected is $r/(n+1)$ if mothers and sisters are equally likely to be affected. Thus the number of affected mothers for that propositus has expected value $r/(n+1)$ and variance $r(n+1-r)/(n+1)^2$, and the expected values and variances can be summed over a group of propositi and compared to the observed number of affected mothers by the following statistic:

$$(|\text{observed} - \text{sum of expecteds}| - 1)^2 / (\text{sum of variances}),$$

which is distributed approximately as chi-squared with one degree of freedom under the null hypothesis. This test is clearly conservative for testing excess prevalence in siblings, since they will be less likely to be reported as affected, being younger than the parents, even if the null hypothesis is true. The degree of excess prevalence in siblings as compared to parents was tested between groups by a stratified analysis, in which the proportions of affected parents were compared in a series of 2×2 tables with fixed r and n and then combined to give a single chi-squared statistic.

Other analyses were done using ordinary chi-squared tests (sex ratios in different groups) or Wilcoxon rank sum tests (ages at onset in different groups).

RESULTS

Satisfactorily completed questionnaires were returned by 227 (97%) diabetics with thyroid disease, 395 (96%) diabetics without thyroid disease and 478 (94%) controls. Of 227 diabetics with thyroid disease,

personal/family history of thyroid disease showed a highly significant difference ($X^2 = 9.92$, $p < 0.01$).

3. Family history of non-insulin-dependent diabetes

A personal or family history of thyroid disease did not significantly influence the probability of a diabetic having a first degree relative with non-insulin-dependent diabetes. Therefore, for the following analyses, all insulin-dependent diabetics, irrespective of thyroid status, were combined, as were all non-insulin-dependent diabetics.

Table 22 shows the reported prevalence of non-insulin-dependent diabetes in relatives of the three study groups. No significant difference was observed among fathers of the three groups. The prevalence of non-insulin-dependent diabetes in mothers of non-insulin-dependent diabetics was greater than those of insulin-dependent diabetics ($X^2 = 10.49$, $p < 0.01$) and controls ($X^2 = 19.08$, $p < 0.001$), which were similar.

The prevalence of non-insulin-dependent diabetes in siblings of non-insulin-dependent diabetics was greater ($X^2 = 11.12$, $p < 0.001$) than that of insulin-dependent diabetics, which was greater ($X^2 = 5.73$, $p < 0.05$) than that of controls. The prevalences of non-insulin-dependent diabetes in children of the study groups were not significantly different.

Consistent female preponderance was noted among non-insulin-dependent diabetic relatives of insulin-dependent diabetics (15 males, 31 females), non-insulin-dependent diabetics (27 males, 40 females) and controls (8 males, 14 females). Table 23 compares the sex ratios of non-insulin-dependent diabetic relatives in different generations for

all three study groups combined. The male to female ratio was greater ($\chi^2 = 6.25$, $p < 0.05$) in children than in siblings and parents combined. Mean age at onset of non-insulin-dependent diabetes in affected relatives was similar in males and females within each generation.

Table 24 compares the observed and expected prevalences of non-insulin-dependent diabetes in parents of the three study groups, based on the observed prevalence in siblings. The observed exceeded the expected prevalence when considering parents of controls ($\chi^2 = 3.85$, $p < 0.05$) but not parents of insulin-dependent or non-insulin-dependent diabetics. No significant difference was observed between the three study groups in respect of the ratio of observed to expected prevalence of non-insulin-dependent diabetes in parents.

DISCUSSION

Evidence for genetic heterogeneity of insulin-dependent diabetes was originally provided by the observation that relatives of diabetics with thyroid antibodies are, themselves, more likely to have thyroid antibodies than those of diabetics without thyroid antibodies (Nissley et al., 1973; Fialkow et al, 1975). This distinction is supported by the present study which shows that diabetics with coexisting thyroid disease are more likely to report a first degree family history of thyroid disease than diabetics without coexisting thyroid disease. Moreover, the familial aggregation of thyroid disease is most evident when considering siblings of the probands. Patients with a personal thyroid history may take a particular interest in the distribution of thyroid disorders in close relatives, and our findings may merely reflect bias in the familial ascertainment rate of thyroid disease. However, it

seems unlikely that a significantly higher ascertainment rate should be selectively operative for siblings. A similarly increased frequency of thyroid disease in siblings (who were predominantly female) compared with other first degree relatives of non-diabetics with Graves' disease has previously been reported (Bartels, 1941; Martin and Fisher, 1945). The best fitting genetic theory to explain this finding was offered to be a 'single-recessive, autosomal type of inheritance with relative limitation to women and a reduced penetrance rate of 70 to 80% in homozygotes' (Bartels, 1941). In the absence of reliable comparative data for non-diabetic hypothyroid patients, it would appear that the coexistence of diabetes has little influence on the familial distribution of thyroid disease. Female preponderance is a feature of affected relatives with thyroid disease as it is of diabetic probands (Chapter 6) and non-diabetic probands (Tunbridge et al., 1977a) with thyroid disease. The relatives of hyperthyroid and hypothyroid propositi seem to be similarly susceptible to both hyper- and hypothyroidism, there being no tendency for either condition to 'breed true'. An increased susceptibility to thyroid disease was observed in the relatives of both insulin-dependent and non-insulin-dependent diabetics with thyroid disease, suggesting that the families of both groups of diabetics were equally predisposed to "autoimmunity". A further proportion of insulin-dependent diabetics having no personal thyroid history have a first degree family history of thyroid disease and may, on this account, also be regarded as having autoimmune diabetes. In spite of the recognised association between clinical thyroid disease and type I diabetes, family studies have indicated that type I diabetes and thyrogastric autoimmunity have differing genetic determinants (Gorsuch et al., 1980). Thus,

it is not surprising that autoimmune type 1 diabetics without thyroid disease may yet have a strong thyroid family history. Non-insulin-dependent diabetics without thyroid disease exhibit a thyroid family history indistinguishable from that of controls, confirming that the aetiologies of non-insulin-dependent diabetes and thyroid disease are unrelated.

Insulin-dependent diabetes is more commonly reported in siblings of autoimmune diabetics than in those of non-autoimmune diabetics. This observation is unlikely to be related to ascertainment bias and therefore represents another clinical feature of autoimmune diabetics which is at variance with those of the general diabetic population. Furthermore, it adds support to the view that inherited factors are strongly implicated in the pathogenesis of autoimmune diabetes as with other autoimmune disorders. When comparing the prevalences of insulin-dependent diabetes in siblings of the study groups, autoimmune non-insulin-dependent diabetics lie between autoimmune and non-autoimmune insulin-dependent diabetics and are significantly different from non-autoimmune non-insulin-dependent diabetics and controls. This suggests that some of the autoimmune non-insulin-dependent diabetics are type 1 diabetics although non-insulin-dependent. Some autoimmune non-insulin-dependent diabetics undoubtedly represent type 2 diabetics having coexisting autoimmune thyroid disease - the inevitable, randomly determined coincidence of two relatively common disorders.

The prevalence of insulin-dependent diabetes in siblings of insulin-dependent diabetics without a personal/family history of thyroid disease was greater than that of controls suggesting that inherited fac-

tors also play a role in the pathogenesis of non-autoimmune insulin-dependent diabetes. This difference might be due to ascertainment bias. However, there was no significant difference in prevalence of insulin-dependent diabetes when comparing the relatives of non-insulin-dependent diabetics without a personal/family history of thyroid disease and controls. The latter finding further serves to confirm that the risks of developing insulin-dependent and non-insulin-dependent diabetes are inherited independently of one another.

As might be predicted from their greater age, the prevalence of insulin-dependent diabetes in parents of non-autoimmune propositi exceeded that of siblings. In contrast, and despite the age difference between parents and siblings, the prevalence of insulin-dependent diabetes in siblings exceeded that of parents of autoimmune propositi, there being a significant difference between autoimmune and non-autoimmune propositi in the ratio of observed to expected prevalence in parents, based on the observed prevalence in siblings. The excessive susceptibility to insulin-dependent diabetes exhibited by siblings of autoimmune diabetic propositi is very similar to the higher prevalence of thyroid disease in siblings of propositi with Graves' disease as reported in this and other studies (Bartels, 1941; Martin and Fisher, 1945). Thus, not only is the heritability of insulin-dependent diabetes increased in siblings of autoimmune diabetics, but also the pattern of inheritance may be distinguished from that found in the general diabetic population and may represent a characteristic feature of organ-specific autoimmune diseases.

Male preponderance in insulin-dependent diabetic offspring of dia-

betic propositi was a feature of both autoimmune and non-autoimmune diabetics. This apparent sex imbalance requires to be confirmed by extending the number of propositi studied and may simply reflect the previously reported (Bloom et al., 1975) male preponderance of the young, insulin-dependent diabetic population whose sex ratio reverses with increasing age at onset (Chapter 9). Accordingly, as the children of diabetic propositi grow older, a greater proportion of daughters than sons may develop insulin-dependent diabetes. Alternatively, these findings might be related to the observation (Cudworth et al., 1979) (as yet unconfirmed) that male children are more likely than female children to inherit the HLA A1-B8 haplotype which is known to confer susceptibility to type 1 diabetes.

The relatives of non-insulin-dependent diabetics had a higher prevalence of non-insulin-dependent diabetes than those of controls and insulin-dependent diabetics, in accordance with previous studies (Irvine et al., 1977c; Gottlieb, 1980; Tattersall and Fajans, 1975). Coexisting thyroid disease in propositi had no influence on the familial pattern of non-insulin-dependent diabetes confirming that thyroid disease and non-insulin-dependent diabetes have unrelated aetiologies. That insulin-dependent diabetics reported a higher prevalence of non-insulin-dependent diabetes in siblings than did controls suggests the possibility that some insulin-dependent propositi were type 2 diabetics and/or that some non-insulin-dependent relatives were type 1 diabetics. Ascertainment bias might equally explain this observation. As with the families of insulin-dependent diabetics, the male preponderance of non-insulin-dependent diabetic children as compared with siblings and parents may be of doubtful significance, particularly in view of the

very small number of children reported to have non-insulin-dependent diabetes. Important environmental influences may also distort the sex ratios of the different generations of relatives, including obesity and parity. Moreover, the familial frequency of non-insulin-dependent diabetes as reported by propositi is likely to underestimate the true prevalence, as has previously been shown (Keen and Track, 1968).

In accordance with other reports, this study confirms that insulin-dependent and non-insulin-dependent diabetes are inherited separately and that some type 1 diabetics (i.e. non-insulin-dependent diabetics with a personal or family history of thyroid disease) are not necessarily insulin-treated. Heterogeneity with respect to the pattern of inheritance of insulin-dependent diabetes clearly exists within the type 1 diabetic population. Autoimmune diabetics demonstrate greater inherited susceptibility to insulin-dependent diabetes than non-autoimmune diabetics, with siblings being at greatest risk. Autoimmune and non-autoimmune type 1 diabetics may thus have differing modes of inheritance of diabetes.

TABLE 16

Comparison of characteristics of participants in the five study groups

	Age (yr)	Duration of Diabetes (yr)	Sex Ratio (M:F)	Siblings	Number of Children
Insulin-dependent diabetics with thyroid disease (n=145)	57 ± 1.1	17 ± 0.9	0.20	2.8 ± 0.2	1.4 ± 0.1
Non-insulin-dependent diabetics with thyroid disease (n=82)	66 ± 1.1	6 ± 0.6	0.19	3.2 ± 0.3	2.5 ± 0.3
Insulin-dependent diabetics without thyroid disease (n=279)	58 ± 0.7	18 ± 0.6	0.90	2.9 ± 0.1	1.6 ± 0.1
Non-insulin-dependent diabetics without thyroid disease (n=116)	64 ± 0.9	7 ± 0.6	0.22	3.0 ± 0.2	1.8 ± 0.2
Controls (n=478)	56 ± 0.4	-	1.14	2.0 ± 0.1	1.4 ± 0.1

Data expressed as mean ± S.E.

TABLE 17

Reported prevalence (%) of thyroid disease in first degree relatives of participants in the five study groups

	<u>Father</u>	<u>Mother</u>	<u>Brother</u>	<u>Sister</u>	<u>Sibling</u>	<u>Son</u>	<u>Daughter</u>
1. Insulin-dependent diabetics with thyroid disease (n=145)	0.7	4.8	3.0	10.7	7.1	0	1.0
2. Non-insulin-dependent diabetics with thyroid disease (n=82)	0	4.9	0.9	10.1	6.1	1.0	7.0
3. Insulin-dependent diabetics without thyroid disease (n=279)	0.3	4.7	0.5	4.5	2.6	0	0.4
4. Non-insulin-dependent diabetics without thyroid disease (n=116)	0	1.7	0	0.6	0.3	0	3.0
5. Controls (n=478)	0.8	2.7	0.2	2.0	1.1	0	0.5
1. vs 2.	NS	NS	NS	NS	NS	NS	NS
1. vs 3.	NS	NS	$x^2 = 4.84,$ $p < 0.05$	$x^2 = 3.91,$ $p < 0.05$	$x^2 = 6.80,$ $p < 0.01$	NS	NS
3. vs 4.	NS	NS	NS	$x^2 = 5.18,$ $p < 0.05$	$x^2 = 4.83,$ $p < 0.05$	NS	NS
3. vs 5	NS	NS	NS	$x^2 = 5.18,$ $p < 0.05$	$x^2 = 6.00,$ $p < 0.05$	NS	NS

TABLE 18

Comparison of observed and expected* prevalences of thyroid disease in
parents of participants in the five study groups

	<u>Observed</u>	<u>Expected</u>	<u>Variance</u>	<u>χ^2</u>	<u>Significance</u>
Insulin-dependent diabetics with thyroid disease	4	9.74	6.29	4.36	p<0.05
Non-insulin-dependent diabetics with thyroid disease	3	4.86	3.06	0.50	NS
Insulin-dependent diabetics without thyroid disease	9	9.75	6.06	0.01	NS
Non-insulin-dependent diabetics without thyroid disease	1	0.28	0.24	0.20	NS
Controls	6	6.75	4.05	0.01	NS

* See text for explanation

TABLE 19

Reported prevalence (%) of insulin-dependent diabetes in first degree relatives of participants in the five study groups

	<u>Father</u>	<u>Mother</u>	<u>Brother</u>	<u>Sister</u>	<u>Sibling</u>	<u>Son</u>	<u>Daughter</u>
1. Insulin-dependent diabetics with personal/family history of thyroid disease (n=179)	1.0	4.5	5.3	7.1	6.3	4.1	0.7
2. Non-insulin-dependent diabetics with personal/family history of thyroid disease (n=87)	2.3	5.7	4.8	3.2	3.9	2.7	0
3. Insulin-dependent diabetics without personal/family history of thyroid disease (n=245)	3.2	5.3	1.8	3.1	2.4	4.7	1.0
4. Non-insulin-dependent diabetics without personal/family history of thyroid disease (n=111)	4.5	2.7	0.6	2.5	1.5	0	0
5. Controls (n=478)	1.5	1.9	0.7	0.4	0.5	0	0
1 vs 2.	NS	NS	NS	NS	NS	NS	NS
1 vs 3.	NS	NS	$x^2 = 6.05,$ $p < 0.05$	NS	$x^2 = 7.71,$ $p < 0.01$	NS	NS
3 vs 4.	NS	NS	NS	NS	NS	NS	NS
3 vs 5.	NS	$x^2 = 7.52,$ $p < 0.01$	NS	$x^2 = 7.00,$ $p < 0.01$	$x^2 = 8.81,$ $p < 0.01$	NS	NS

TABLE 20

Sex ratio of insulin-dependent diabetic relatives of
autoimmune and non-autoimmune propoiti

	Autoimmune propoiti*		Non-autoimmune propoiti†	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
Parents	4 (42)	13 (52)	20 (57)	25 (53)
Siblings	19 (33)	25 (35)	11 (42)	17 (46)
Children	8 (16)	1 (14)	9 (12)	2 (9)

Mean age at onset of diabetes in parenthesis

* Insulin-dependent and non-insulin-dependent diabetics with personal/family history of thyroid disease.

† Insulin-dependent and non-insulin-dependent diabetics without personal/family history of thyroid disease, and controls.

TABLE 21

Comparison of observed and expected* prevalences of insulin-dependent diabetes in parents of the participants in the five study groups

	<u>Observed</u>	<u>Expected</u>	<u>Variance</u>	<u>χ^2</u>	<u>Significance</u>
Insulin-dependent diabetics with personal/family history of thyroid disease	8	13.48	6.76	3.67	NS
Non-insulin-dependent diabetics with personal/family history of thyroid disease	6	5.8	3.42	0	NS
Insulin-dependent diabetics without personal/family history of thyroid disease	15	10.35	6.02	2.86	NS
Non-insulin-dependent diabetics without personal/family history of thyroid disease	6	4.17	2.36	0.75	NS
Controls	11	6.17	3.59	5.22	p < 0.05

* See text for explanation

TABLE 22

Reported prevalence (%) of non-insulin-dependent diabetes
in first degree relatives of insulin-dependent diabetics,
non-insulin-dependent diabetics and controls

	<u>Father</u>	<u>Mother</u>	<u>Brother</u>	<u>Sister</u>	<u>Sibling</u>	<u>Son</u>	<u>Daughter</u>
1. Insulin-dependent diabetics (n=424)	1.1	3.0	1.5	2.8	2.2	0.3	0
2. Non-insulin-dependent diabetics (n=198)	3.0	9.6	6.1	6.6	6.4	1.4	0
3. Controls (n=478)	1.2	1.9	0.2	0.9	0.5	0.2	0
1. vs 2.	NS	$x^2 = 10.49,$ $p < 0.01$	$x^2 = 9.74,$ $p < 0.01$	NS	$x^2 = 11.12,$ $p < 0.001$	NS	NS
1. vs 3.	NS	NS	NS	NS	$x^2 = 5.73,$ $p < 0.05$	NS	NS
2. vs 3.	NS	$x^2 = 19.08,$ $p < 0.01$	$x^2 = 13.14,$ $p < 0.001$	$x^2 = 7.02,$ $p < 0.01$	$x^2 = 23.56,$ $p < 0.001$	NS	NS

TABLE 23

Sex ratio of non-insulin-dependent diabetic relatives of all participants

	<u>Male</u>	<u>Female</u>
Parents	17 (67)	41 (64)
Siblings	28 (57)	44 (57)
Children	5 (38)	0

Mean age at onset of diabetes in parenthesis

TABLE 24

Comparison of observed and expected* prevalences of non-insulin-dependent diabetes in parents of insulin-dependent diabetics, non-insulin-dependent- diabetics and controls

	<u>Observed</u>	<u>Expected</u>	<u>Variance</u>	<u>x²</u>	<u>Significance</u>
Insulin-dependent diabetics	14	13.13	7.67	0.02	NS
Non-insulin-dependent diabetics	16	16.37	9.47	0.00	NS
Controls	11	6.65	3.85	3.85	p < 0.05

PART 5

Summary

Insulin-dependent diabetes and autoimmune thyroid disease show similar histological, immunological and HLA antigen characteristics. Animal models of each condition arise spontaneously and concomitantly, and may be induced by immunisation with pancreatic islet and thyroid tissue, respectively.

This thesis shows that the prevalence of clinically recognised autoimmune thyroid disease is three times more common amongst insulin-dependent than amongst non-insulin-dependent diabetics. Similarly, clinically unrecognised primary autoimmune thyroid failure, as evidenced by elevation of the serum thyrotrophin concentration, is twice as common amongst insulin-dependent than amongst non-insulin-dependent diabetics. Elevation of the serum thyrotrophin concentration is usually accompanied by thyroid microsomal antibodies. In antibody positive patients with an elevated thyrotrophin concentration but serum total thyroxine concentration within the normal range, hypothyroidism develops at a rate of 5% per annum. These observations suggest that autoimmune thyroid disease and insulin-dependent diabetes may share a common aetiology. In patients suffering from both diabetes and autoimmune thyroid disease, the two conditions are often diagnosed concomitantly suggesting both a common and coincident pathogenesis.

Autoimmune thyroid disease represents a heterogeneous group of diseases including Hashimoto's thyroiditis, primary atrophic hypothyroidism and Graves' disease each having distinct clinical, histological,

immunological and HLA antigen characteristics. Similarly, within the insulin-dependent diabetic population, patients with coexisting autoimmune thyroid disease differ from patients without coexisting autoimmune thyroid disease by exhibiting marked female preponderance, late age at onset of diabetes, increased frequency of HLA-B8 and of cytoplasmic and complement fixing islet cell antibodies. Thus, clinical HLA antigen and immunological heterogeneity is a feature both of insulin-dependent diabetes and autoimmune thyroid disease.

Identical twin studies of insulin-dependent diabetes show a concordance rate of 50% suggesting the aetiology of this condition to be dependent both upon inherited and environmental factors. Further evidence for an environmental precipitant to the development of insulin-dependent diabetes derives from the peak ages of incidence of this condition, which may be associated with exposure to new infective agents, together with its variable seasonal incidence. This thesis has confirmed the variable seasonal incidence of male, teenage, insulin-dependent diabetes. This observation is in accordance with the reported predilection of environmentally induced experimental diabetes for male animals. In contrast, no such seasonal variation in incidence nor peaks in age incidence were observed in insulin-dependent diabetics with coexisting thyroid disease suggesting their diabetic aetiology to be independent of the environment. Conversely, a personal or family history of thyroid disease in a diabetic increases the chance of that patient having an insulin-dependent diabetic sibling, suggesting that inherited factors may play a predominant role in the aetiology of diabetes when associated with thyroid disease. It is therefore proposed that environmental factors are largely responsible for the development

of insulin-dependent diabetes in younger, male patients, while inherited factors, possibly leading to autoimmunity, may predispose to insulin-dependent diabetes in the older, female patients who have coexisting autoimmune thyroid disease.

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