

Gastric, haematological, and immunological abnormalities in Hashimoto's thyroiditis¹

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EDITORIAL COMMENT Reduced gastric acidity and intrinsic factor production are common in Hashimoto's thyroiditis. This cannot be explained on the basis of changes with age but may be related to the presence of gastric parietal cell antibodies in the blood.

The association of pernicious anaemia with various forms of thyroid disease (Irvine, Davies, Delamore, and Williams, 1962; Tudhope and Wilson, 1962; Markson and Moore, 1962; Irvine, 1963; Doniach and Roitt, 1964; Irvine, 1965; Schiller, Spray, Wangel, and Wright, 1965; Schiller, 1965; Ardeman, Chanarin, Krafchik, and Singer, 1966) suggests a common aetiological or pathogenetic mechanism. Circulating autoantibodies are often found in both types of disorder (Doniach and Roitt, 1963; Roitt, Doniach, and Taylor, 1963) and an inflammatory, frequently atrophic, process occurs in the thyroid gland in Hashimoto's thyroiditis (Doniach and Roitt, 1963) and in the gastric mucosa in pernicious anaemia (Doniach and Roitt, 1964; Irvine, 1965). Hence, both disorders may have an autoimmune basis. Among patients with thyroid abnormalities, those with Hashimoto's thyroiditis display manifestations of thyroid autoimmunity with the greatest frequency, and titres of thyroid autoantibodies are higher in this disorder than in other types of thyroid disease (Doniach and Roitt, 1963; Doniach and Roitt, 1964).

By means of the augmented histamine test, it has been shown that there is an increased incidence of achlorhydria in both hyperthyroidism (Williams and Blair, 1964) and primary hypothyroidism (Tudhope and Wilson, 1962), and the maximal acid output in hyperthyroidism is less than in normal subjects (Williams and Blair, 1964). Gastric function has been investigated in 'chronic thyroiditis' (Irvine, 1965), and also in isolated cases of Hashimoto's thyroiditis (Adams, Glen, Kennedy, Mackenzie, Morrow,

Anderson, Gray, and Middleton, 1964; Jeffries, Todd, and Slesinger, 1966; Ardeman *et al.*, 1966).

Since gastric secretory function has not been assessed in a larger group of patients with Hashimoto's thyroiditis, it seemed of interest to perform certain gastric studies and to attempt to correlate the results with specific immunological and haematological abnormalities that may be present.

MATERIALS AND METHODS

PATIENTS Eighteen patients with Hashimoto's thyroiditis were selected on a basis of availability from those attending the Thyroid Clinic of the Massachusetts General Hospital. The original diagnosis had been made on clinical grounds, with or without evidence of thyroid dysfunction, and had been confirmed histologically in 14 patients; in four the clinical diagnosis was supported by past tanned red-cell agglutination tests giving titres of 1/2,560 or higher. The mean age of the patients studied was 41.4 (range, 17-60); all were females.

Clinical data on these patients are summarized in Table I. At the time of diagnosis, seven were considered hypothyroid and two hyperthyroid. When reviewed in connexion with this study 15 were receiving thyroid extract either to correct hypothyroidism or to reduce goitre size. None had been rendered hypermetabolic by thyroid replacement therapy. Two were myxoedematous (cases 16 and 18); the former (case 16) last attended the Clinic three years ago and had discontinued treatment. The remaining patients were euthyroid. Since specific therapy and a return to the euthyroid state do not consistently alter gastric acidity (Tudhope and Wilson, 1962; Williams and Blair, 1964) and antibody titres (Owen and Smart, 1958; Fulthorpe, Roitt, Doniach, and Couchman, 1961), we are assuming that gastric function in our patients had not been affected by therapy.

METHODS Haematocrits were estimated on venous blood; a level of 38% was considered the lower limit of

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TABLE I
CLINICAL FINDINGS IN 18 PATIENTS WITH HASHIMOTO'S
THYROIDITIS¹

Case No.	Age	At Diagnosis		At Review	
		Histology	T.R.C. ²	Thyroid Function	Thyroid Therapy
1	23	+		Normal	+
2	42	+		Normal	+
3	55	+		Hypo-thyroid	+
4	53	+		Normal	+
5	24	+		Slightly hypo-thyroid	+
6	17	+		Slightly hypo-thyroid	+
7	53	+		Hypo-thyroid	+
8	60	+		Normal	+
9	30	+		Toxic	0
10	55		1/2,560	Normal	+
11	43		1/40,000	Hypo-thyroid	+
12	42	+		Normal	+
13	39	+		Normal	+
14	43		1/20,000	Normal	+
15	34		1/2,560,000	Hypo-thyroid	+
16	46	+		Normal	0
17	37	+		Toxic	0
18	50	+		Hypo-thyroid	+

¹All patients were female.

²Titre of tanned red-cell agglutination for thyroglobulin antibodies.

normal. Peripheral blood smears were treated with Wright's stain and reviewed by an independent observer. Serum vitamin B₁₂ levels were determined by the method of Rosenthal and Sarett (1952) using *Lactobacillus leichmannii* as the test organism; in this laboratory, the lower limit of normal, established in a large survey of normal subjects, is 200 µg./ml. Vitamin B₁₂ absorption was measured by the Schilling test (Schilling, 1953), an excretion of 7% or more of the ingested radioactivity in the 24 hours following administration being considered normal.

Gastric secretion of acid and intrinsic factor was studied by the augmented histamine test (Kay, 1953) using a fluoroscopically positioned plastic Levine tube. The volume of gastric juice, total HCl secreted (in mEq.), designated maximal acid output, and maximal pH change during the post-histamine hour were determined. Hypochlorhydria was considered to be present when the maximal acid output was less than 10 mEq./hour (Irvine, Davies, Teitelbaum, Delamore, and Williams, 1965). A diagnosis of achlorhydria was made when it was 0 mEq./hour (Bock, Richards, and Witts, 1963) or when the pH failed to drop below 6.0 (Marks, Bank, Moshal, and Louw, 1963).

For the estimation of intrinsic factor secretion, gastric juice obtained by the augmented histamine test was promptly neutralized to pH 7.0, filtered through cheese cloth, chilled to -20°C., and stored at that temperature.

The assay was performed by the method of Ardeman and Chanarin (1963) as modified by Gottlieb, Lau, Wasserman, and Herbert (1965). It was not feasible or desirable in the present study to use normal subjects as controls. However, in an attempt to compare the results of estimations of intrinsic factor secretion in our hands with the results obtained by others, 10 male patients (mean age, 42.9; range, 29-64) with radiologically-proven duodenal ulceration, before surgery, were studied in place of a normal control group and underwent the augmented histamine test. As will be noted below, mean intrinsic factor production and concentration in the ulcer patients was comparable with the means observed by Ardeman, Chanarin, and Doyle (1964) in 10 male patients (mean age, 38; range, 19-60) with active duodenal ulceration and in nine male normal subjects (mean age, 21; range, 19-21).

Antibodies to intrinsic factor in serum were assayed by extensions of the methods used to assay intrinsic factor in gastric juice (Ardeman and Chanarin, 1963; Gottlieb *et al.*, 1965). Circulating antibodies to thyroglobulin, to the second colloidal antigen, and to the cytoplasmic antigen were detected by a modification (Valotton, Pretell, and Forbes, 1967) of the indirect immunofluorescent method of Balfour, Doniach, Roitt, and Couchman (1961). For the titration of antibody to thyroglobulin the passive haemagglutination test was employed, using thyroglobulin-coated tanned red cells according to a modification (Fulthorpe *et al.*, 1961) of the method of Boyden (1951); anti-microsomal thyroid antibodies (Roitt and Doniach, 1958; Belyavin and Trotter, 1959) were assayed with the complement-fixation test. Gastric parietal-cell antibodies in serum were sought by the indirect immunofluorescent method (Balfour *et al.*, 1961).

PROCEDURE

The nature and purpose of the study was explained to each patient and consent was obtained. Patients were investigated as outpatients to minimize the time spent away from home or work. Those with hypertension or a history of allergy were excluded. On the first visit, the augmented histamine test was performed and gastric juice was analysed for volume, HCl, and intrinsic factor content. Examinations were made of haematocrit, peripheral blood smear, serum vitamin B₁₂ level, and gastric parietal-cell, thyroid, and intrinsic factor antibodies. The Schilling test was performed on a subsequent visit.

RESULTS

Observations made on the patients with Hashimoto's thyroiditis are summarized in Table II.

The mean haematocrit was 39.6% (range, 35-47). Six patients had haematocrit levels below 38%. Red cells, white cells, and platelets were morphologically normal in all patients. The mean serum vitamin B₁₂ level was 223 µg./ml. (range, 90-360). Ten patients had serum vitamin B₁₂ levels within the normal range; eight had levels below 200 µg./ml. The

TABLE II

LABORATORY FINDINGS IN 18 PATIENTS WITH HASHIMOTO'S THYROIDITIS

Case No.	Haemato-crit (%)	Serum Vitamin B ₁₂ (μg./ml.)	Schilling Test (% radio-activity excreted)	Gastric Juice ¹			Thyroid Antibodies				Gastric Antibodies				
				Volume (ml.)	Maximal Acid Output (mEq.)	Change in pH		Intrinsic Factor Amount (units)	Concentration (units/ml.)	Cyto-plasmic	Colloid Titre	T.R.C. ² Titre	C.F. ³ Titre	Parietal Cell	Intrinsic Factor
						From	To								
1	38	360	20.0	224	11.6	6.1	1.6	7,034	31	+	+	1/10	1/10	+	—
2	42	360	33.0	190	10.0	1.5	1.3	4,826	25	+++	+++	1/20,480	1/80	—	—
3	42	230	25.8	150	14.6	1.7	1.2	4,483	30	+++	++	1/40,960	1/160	—	—
4	37	160	28.0							++	+++	1/1,310,000		—	—
5	42	255	34.9	67	3.5	2.0	1.7	1,707	24	++	—		1/10	—	—
6	37	325	15.2	122	10.6	2.2	1.1	1,474	12	++	+++	1/20,480		—	—
7	37	210		60	1.8	1.9	1.4	1,680	28	+++	+++	1/10,240	1/160	++	—
8	39	310	30.1	120	15.3	1.5	1.0	1,824	15	+++	+	1/20	1/320	—	—
9	40	135	22.0	47	3.1	1.6	1.2	865	18	—	—	1/10		—	—
10	42	310	33.0	139	11.2	2.4	1.1	4,587	35	++	+++	1/320	1/80	—	—
11	41	120	20.6	162	16.4	1.9	1.2	6,564	41	++	+++	1/20,480	1/40	—	—
12	42	175	23.6	146	14.6	1.6	1.1	5,256	36	—	—			—	—
13	47	90		127	9.6	1.7	1.2	2,794	22	++	+++	1/20,480	1/160	—	—
14	41	225	23.4	103	3.6	6.5	1.6	1,751	17	—	++	1/40,960		—	—
15	37	190	26.1	64	3.8	2.1	1.4	1,088	17	++	+	1/1,310,000	1/1,280	—	—
16	35	250	14.2	69	1.5	3.0	1.2	1,050	15	++	++	1/1,310,000	1/160	—	—
17	39	155	25.5	69	5.7	1.9	1.2	1,311	19	++	—		1/640	+	—
18	35	145		60	2.3	5.8	1.4	900	15	++	—	1/80	1/160	+	—

¹Specimens obtained during post-histamine hour in augmented histamine test.

²Titre of tanned red-cell agglutination test for thyroglobulin antibodies.

³Titre of complement fixing thyroid microsomal antibodies.

results of the Schilling test, performed in 15 patients, averaged 22.9% (range, 14.2-34.9). Excretion of radioactivity was within normal limits in every patient.

The augmented histamine test was performed in 17 patients. Mean maximal acid output was 8.2 mEq./hour (range, 1.5-16.4). Hypochlorhydria was present in nine patients; none had achlorhydria. Mean gastric juice volume in the post-histamine hour was 113 ml. (range, 47-224). Mean production of intrinsic factor of the patients in the post-histamine hour was 2,984 units (range, 865-7,034), the mean concentration being 23 units/ml. (range, 12-41). The patients with Hashimoto's thyroiditis showed a lower mean intrinsic factor production and concentration than the patients with duodenal ulceration, whose mean intrinsic factor production was 11,000 units/hour (range, 6,400-17,800) and concentration 46 units/ml. (range, 40-51). The results in the ulcer group correspond closely to those reported by Ardeman, Chanarin, and Doyle (1964) in patients with duodenal ulcer (mean intrinsic factor concentration 51 units/ml., range, 26-83; mean intrinsic factor production 8,600 units/hour, range, 2,700-14,000) and are similar to the results they obtained in normal subjects (mean intrinsic factor concentration 56 units/ml., range, 14-147; mean intrinsic factor production 8,900 units/hour, range, 2,200-18,300). Although the age and sex distribution of each group of patients is different, and thus the various groups are not

directly comparable, it appears nevertheless that the present group with Hashimoto's thyroiditis secretes diminished amounts of intrinsic factor.

None of the 18 patients with Hashimoto's thyroiditis had antibodies to intrinsic factor. Moderately to strongly positive tests for circulating gastric parietal-cell antibodies were found in four patients (22.2%). Thyroid antibodies against colloidal antigens were found in 13 patients (72.2%). Twelve patients (66.6%) had a significant raised tanned red cell agglutination titre, the titre in 10 patients equalling or exceeding 1/10,240. Thyroid cytoplasmic antibodies were found in the sera of 15 patients (83.3%) and 11 (61.2%) had a complement-fixation titre of 1/40 or higher. In sum, 16 patients (88.8%) had significantly elevated titres of antibodies to thyroid antigens. It is noted, however, that four of the 18 patients were chosen for study in part on the basis of known high tanned red cell agglutination titres. Of the remaining 14 patients, eight (57.1%) had an elevated titre, eight (57.1%) had antibodies against colloidal antigens, 12 (85.7%) had thyroid anti-cytoplasmic antibodies, and eight (57.1%) had complement-fixation titres of 1/40 or above. Thus, of the 14 patients with histological evidence of Hashimoto's thyroiditis, 12 (85.7%) had one or more of the thyroid antibodies. These results are similar to those reported in the literature (Doniach and Roitt, 1963; Irvine, Davies, and Sumerling, 1965; Doniach, Nilsson, and Roitt, 1965).

DISCUSSION

This study was undertaken to investigate various haematological, gastric, and immunological abnormalities in patients with Hashimoto's thyroiditis. None were found to have pernicious anaemia or latent pernicious anaemia as defined by Wangel and Schiller (1966). This is in keeping with the findings of Mulhern, Masi, and Shulman (1966), who showed that patients with coexisting pernicious anaemia would seem to represent the exceptional case of Hashimoto's thyroiditis. However, in the present series of patients certain abnormalities were detected, and it is of interest to consider their possible significance.

Vitamin B₁₂ deficiency, which may occur in the absence of anaemia (Spray, 1962; Henderson, Strachan, Beck, Dawson, and Daniel, 1966; Wangel and Schiller, 1966), was demonstrated in cases 4, 9, 11, 12, 13, 15, 17, and 18. The morphological abnormalities of the red and/or white cell series commonly observed in vitamin B₁₂ deficiency were not found in our patients. It is recognized that low serum vitamin B₁₂ levels may be found in association with normal vitamin B₁₂ absorption (Schiller, 1965; Henderson *et al.*, 1966) and that this may occur even in atrophic gastritis (Whiteside, Mollin, Coghill, Williams, and Anderson, 1964) in which secretion of intrinsic factor is often reduced (Ardeman and Chanarin, 1966). An analysis of variance concerning the relation of intrinsic factor secretion to serum vitamin B₁₂ levels and the results of the Schilling tests showed the regressions to be not significant ($P > 0.05$). Ardeman and Chanarin (1965), however, have shown that the Schilling test gives abnormal results only when intrinsic factor secretion is 500 units or less during the post-histamine hour. All of our patients secreted an amount greater than this. Our results confirm that the Schilling test is insufficiently sensitive in the detection of anything other than a gross deficiency of intrinsic factor secretion and vitamin B₁₂ absorption.

Estimates of the range of maximal acid output in normal female subjects differ. Williams and Blair (1964) reported a range of 7.07-20.50 mEq./hour, while Marks *et al.* (1963) reported a wider range of 0.1-30.0 mEq./hour. The mean values for maximal acid output taken from four series quoted by Williams and Blair (1964) varied from 13.93 to 17.70 mEq./hour and showed good agreement. The mean for our series of patients was 8.2 mEq./hour (range, 1.5-16.4). These data strongly suggest that our patients showed a tendency to reduction in acid production in comparison with normal subjects. Tudhope and Wilson (1962) found that almost 50% of their patients with primary myxoedema had

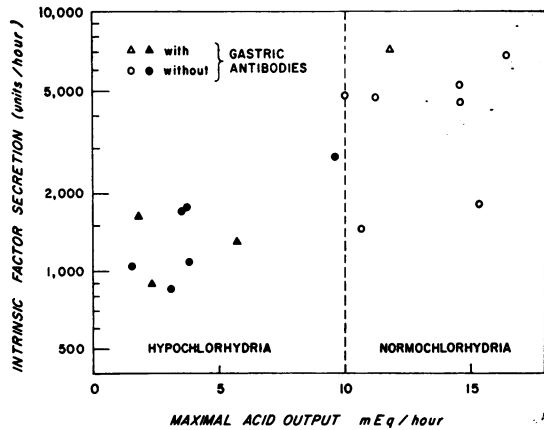


FIG. 1. Secretion of acid and intrinsic factor in 17 patients with Hashimoto's disease in the post-histamine hour.

achlorhydria. Irvine (1965) studied gastric secretion in a group of 73 patients with 'chronic thyroiditis' and found that 62 had maximal acid output levels below 10 mEq./hour, while 30 had achlorhydria. Since Irvine did not distinguish between primary myxoedema and Hashimoto's thyroiditis, we cannot directly compare our results with his. However, it appears that achlorhydria and hypochlorhydria are less common in our patients than in subjects with primary myxoedema or 'chronic thyroiditis'.

Irvine (1965) demonstrated a positive correlation in a heterogeneous group of patients between the quantity of acid and of intrinsic factor secreted in the post-histamine hour. A simple regression line relating intrinsic factor secretion to acid secretion and volume of gastric juice was computed for our patients. The slopes of this bivariate regression line were highly significant ($P < 0.0005$). Furthermore, acid secretion and volume of gastric juice were significantly correlated ($P < 0.00005$). In man, intrinsic factor and HCl are believed to be secreted by the parietal cells (Hoedemaker, 1965) which are reduced in number in gastritis and absent in gastric atrophy (Bock *et al.*, 1963). Thus, atrophic gastritis or gastric atrophy was probably present in some of our patients with Hashimoto's thyroiditis.

Atrophic gastritis is more common in patients whose serum contains antibodies to gastric parietal cells (Doniach and Roitt, 1964; Coghill, Doniach, Roitt, Mollin, and Williams, 1965). The reported incidence of gastric parietal-cell antibodies in Hashimoto's thyroiditis is 25-30% (Roitt *et al.*, 1963; Irvine *et al.*, 1965). In our series of 18 cases these antibodies were detected in four patients, an incidence of 22.2%. One patient (case 1) with gastric parietal-cell antibodies secreted 11.6 mEq. acid in

the post-histamine hour, but the volume of gastric juice produced and the quantity of intrinsic factor secreted were the highest in this series of patients. However, each of the other patients with gastric parietal-cell antibodies (cases 7, 17, and 18) had hypochlorhydria, secreted less than 1,700 units intrinsic factor/hour and produced small volumes of gastric juice (Fig. 1). There are too few patients to deduce statistically significant results from this aspect of the study, but it appears that a majority of the patients with gastric parietal-cell antibodies had impaired gastric secretory activity. All of our patients with gastric parietal-cell antibodies were found to have one or more of the circulating thyroid antibodies.

On the basis of diminished acid and intrinsic factor secretion, the patients could be divided into two subgroups (Fig. 1). There was no significant difference between the mean ages of the patients with hypochlorhydria (mean, 39 years) and those with normal acid secretion (mean, 42 years). We stress this point as it has been shown in normal subjects (Marks *et al.*, 1963) and in patients with thyrotoxicosis (Williams and Blair, 1964) that diminished acid production is more common in the older age groups. Thus in our patients, age was probably not the major factor in the development of impaired gastric function. It was further noted that the mean serum vitamin B₁₂ level of the hypochlorhydric group was 184 $\mu\mu\text{g./ml.}$ while that of the normochlorhydric patients was 274 $\mu\mu\text{g./ml.}$

In conclusion, we have confirmed the work of others regarding the incidence of thyroid and gastric parietal-cell antibodies in Hashimoto's thyroiditis. In addition we have shown that hypochlorhydria, diminished intrinsic factor secretion and decreased serum vitamin B₁₂ levels are common, and that gastric parietal-cell antibodies are found more frequently in patients with hypochlorhydria. None of our patients had latent or clinical pernicious anaemia. But, in view of the separation of our patients into two groups, it appears that only some patients with Hashimoto's thyroiditis have associated defects of gastric mucosal function. It is in this group of patients that pernicious anaemia may eventually develop. However, only a longitudinal study of a larger group of patients will validate this hypothesis.

SUMMARY

Eighteen patients with Hashimoto's thyroiditis were studied with respect to gastric, haematological, and immunological abnormalities. Minor abnormalities of haematocrit and serum vitamin B₁₂ level were common. Vitamin B₁₂ absorption was measured by the Schilling test (15 patients) and was normal.

Seventeen patients underwent an augmented histamine test; nine had hypochlorhydria but achlorhydria was not detected. The mean maximal acid output was 8.2 mEq./hour (range, 1.5-16.4). Intrinsic factor production was measured during the post-histamine hour in 17 patients. Mean intrinsic factor production was 2,984 units/hour (range, 865-7,034). Mean intrinsic factor concentration was 23 units/ml. (range, 12-41). Sixteen patients (88.8%) had significant titres of thyroid antibodies; four (22.2%) had gastric parietal-cell antibodies. None of the patients had serum intrinsic factor antibodies.

It is concluded that reduced gastric acidity and intrinsic factor production are common in Hashimoto's thyroiditis and that these abnormalities are commoner in patients who have gastric parietal-cell antibodies. The question is raised whether all patients with Hashimoto's thyroiditis are equally at risk with regard to the development of gastric secretory hypofunction.

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