SERUM PROTEINS IN THYROID DISEASE

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Abnormalities in the serum proteins have been described in several disorders of thyroid function (Man *et al.*, 1940; Lewis and McCullagh, 1944; Lamberg and Gräsbeck, 1955) and in particular in auto-immune thyroiditis, where a raised serum γ-globulin has been found (Fromm *et al.*, 1953; Luxton and Cooke, 1956; Skillern *et al.*, 1956; Doniach and Hudson, 1957; Greene *et al.*, 1958; Roitt *et al.*, 1958; Hetz *et al.*, 1959; Shulman *et al.*, 1960). The diagnostic significance of these changes is difficult to evaluate because in most studies the number of patients has been small. Furthermore, the control series of normal subjects and of patients with other forms of thyroid disease has been inadequate for statistical evaluation.

In this paper we report a detailed study of the serum protein abnormalities in 182 patients with different thyroid disorders and compare them with those found in 26 normal controls. In addition the diagnostic value of the serum flocculation tests and the erythrocyte sedimentation rate (E.S.R.) has been studied, since both have been suggested as diagnostic aids in auto-immune thyroiditis (Luxton and Cooke, 1956; Doniach and Hudson, 1957; Hubble, 1959).

Materials and Methods

Serum protein determinations were carried out on 26 normal controls and 198 patients with thyroid disease listed in Table I. The latter included: 41 with auto-immune thyroiditis; 40 with primary hypothyroidism; 33 with simple non-toxic goitre; 69 with thyrotoxicosis, including 16 with exophthalmic ophthalmoplegia and 16 euthyroid after treatment; and 15 with thyroid neoplasm. Serum flocculation tests were carried out on 420 patients with thyroid disease, including 41 with auto-immune thyroiditis; 55 with primary hypothyroidism; 127 with simple non-toxic goitre; 181 with thyrotoxicosis, including 15 with exophthalmic ophthalmoplegia; and 16 with thyroid neoplasm (Table II).

The diagnosis of auto-immune thyroiditis (Buchanan *et al.*, 1961), simple non-toxic goitre (Koutras *et al.*, 1960), primary hypothyroidism, and thyrotoxicosis (Crooks *et al.*, 1959; Wayne, 1960) was based on methods described elsewhere. The diagnosis of thyroid neoplasm was confirmed in all patients by histological examination. Patients with systemic disease, which might have been associated with changes in the serum proteins, were excluded from the study.

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Serum protein estimations were carried out by the biuret method of Gornall *et al.* (1949). Filter-paper electrophoresis was performed on serum, using the horizontal-strip method; after staining, quantitative evaluation of the protein pattern was carried out, using an E.E.L. scanner. The percentage value thus found was expressed as g./100 ml. of serum from the total value of the serum globulin.

The serum flocculation tests were carried out by the standard laboratory procedures, and included thymol turbidity (Maclagan, 1944) and flocculation (Maclagan, 1947), zinc sulphate turbidity (Kunkel, 1947), cephalin-cholesterol (Hanger, 1939), and colloidal gold (Prunty *et al.*, 1959) reactions. The E.S.R. was performed by the method of Westergren (1921).

Tests for thyroid auto-antibodies were carried out by both precipitin and complement-fixation methods (Anderson *et al.*, 1959).

Results

The results of the serum protein determinations are summarized in Table I, giving mean values and standard deviations for each group, and the serum flocculation tests and the E.S.R. in Table II.

Auto-immune Thyroiditis

The mean serum albumin in the patients with auto-immune thyroiditis was 4.2 g./100 ml. (S.D. $\pm$0.4), which was significantly lower ($P<0.001$) than the mean value of 5.2 g./100 ml. (S.D. $\pm$0.4) found in the normal subjects. The mean serum globulin of 2.4 g./100 ml. (S.D. $\pm$0.6) in the former was significantly higher ($P<0.001$) than the normal mean value of 1.9 g./100 ml. (S.D. $\pm$0.3). This increase in the total globulins was entirely due to an increase in the γ-globulin fraction ($P<0.001$). Of the 36 patients in whom electrophoresis was performed, 22 (61%) had γ-globulin values which exceeded the highest value (1 g./100 ml.) found in the normal group.

The various serum flocculation tests were abnormal in 56 to 82% of the patients with auto-immune thyroiditis (Table II) and the E.S.R. (23 mm./hr.) was raised. There was a correlation between the thymol turbidity results and the levels of γ-globulin ($r=+0.385$, $P=0.02$).

The effect of thyroxine sodium 0.2 mg. daily was studied on the biochemical abnormalities in 19 patients after a follow-up period of 2 to 36 months (mean follow-up of 15.4 months) and the results are summarized in Table III. There was a highly significant ($P<0.001$) increase in the serum albumin (mean=0.36 g./100 ml.) and a marked ($P<0.001$) decrease in the serum globulin (mean=0.32 g./100 ml.) and γ-globulin values (mean 0.41 g./100 ml.). Furthermore, there was a significant fall in the thymol turbidity ($P<0.001$) and flocculation tests ($P<0.01$) and in the E.S.R. values ($P<0.01$).

Primary Hypothyroidism

The mean serum albumin in the patients with primary hypothyroidism was 4.3 g./100 ml. (S.D. $\pm$0.4), which was significantly lower ($P<0.001$) than the normal mean value of 5.2 g./100 ml. (S.D. $\pm$0.4). The total serum globulin, on the other hand, was only slightly increased, although there was a suggestive increase in the β- ($P<0.1$) and γ- ($P<0.1$) globulins. The trends were present to a greater extent in patients with a positive precipitin test, but the small number (eight patients)

does not permit statistical analysis. Seven of the 40 patients (17%) had a serum γ-globulin value which exceeded 1 g./100 ml.

The various serum flocculation tests were positive in 12.7 to 53.7% of the patients (Table II) and the mean E.S.R. (19 mm./hr.) was increased. The finding of a raised E.S.R. in some patients with primary hypothyroidism is in agreement with the observations of McAlpine (1955) and Lillington *et al.* (1959).

Simple Non-toxic Goitre

The mean serum albumin was 4.9 g./100 ml. (S.D. ±0.5) in patients with simple non-toxic goitre, which was slightly but significantly ($P<0.02$) lower than the mean value of the control series. The total globulins and the different globulin fractions did not, however, differ significantly from the normal. Only 2 of the 33 patients (6%) had γ-globulin levels exceeding 1 g./100 ml.

The serum flocculation tests were abnormal in only a few patients, with the exception of the cephalin cholesterol, zinc sulphate turbidity, and colloidal gold tests, which were abnormal in 26.7%, 36.4%, and 51.9% respectively (Table II). The E.S.R. (mean=7 mm./hr.) lay usually within the normal range of 0 to 12 mm./hr.

Thyrotoxicosis

The mean serum albumin (4.1 g./100 ml., S.D. ±0.4) was more markedly decreased ($P<0.001$) in this disease than in any other thyroid disorder (Table I). The total serum globulins were normal; but there was a slight increase in the γ-globulins ($P<0.1$). Four of the 37 patients (11%) had γ-globulin values exceeding 1 g./100 ml.

The serum flocculation tests were abnormal in a high proportion of the patients—in particular the zinc sulphate turbidity (36.9%), the cephalin-cholesterol (43.4%), and colloidal gold (58%) tests (Table II).

The E.S.R. (mean=6 mm./hr.) was usually within the normal range.

Patients with thyrotoxicosis complicated by severe exophthalmos had similar abnormalities to those found in uncomplicated thyrotoxicosis and do not merit separate consideration.

TABLE I.—*Results of Determinations and Electrophoresis of Serum Proteins in Thyroid Disease*

Clinical Group	Total No. of Cases	Albumin (g./100 ml.)		Globulin (g./100 ml.)		Globulin Fractions (g./100 ml.)							
						a_1		a_2		β		γ	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Normals	26	5·2	±0·4	1·9	±0·3	0·20	±0·05	0·43	±0·09	0·55	±0·11	0·74	±0·15
Auto-immune thyroiditis	41	4·2	±0·4	2·4	±0·6	0·20	±0·09	0·44	±0·13	0·57	±0·12	1·25	±0·50
Primary hypothyroidism	40	4·3	±0·4	2·0	±0·5	0·18	±0·07	0·40	±0·18	0·65	±0·24	0·83	±0·23
Simple goitre	33	4·9	±0·5	1·9	±0·3	0·20	±0·12	0·40	±0·09	0·51	±0·10	0·79	±0·14
Thyrotoxicosis	37	4·1	±0·4	1·9	±0·4	0·18	±0·04	0·37	±0·17	0·52	±0·13	0·82	±0·19
Thyrotoxicosis with exophthalmic ophthalmoplegia	16	4·1	±0·4	1·9	±0·4	0·20	±0·06	0·43	±0·16	0·50	±0·08	0·82	±0·19
Treated thyrotoxicosis	16	4·7	±0·4	1·8	±0·2	0·19	±0·04	0·37	±0·15	0·50	±0·07	0·77	±0·19
Thyroid neoplasm	15	4·5	±0·6	1·9	±0·2	0·21	±0·05	0·50	±0·10	0·46	±0·06	0·78	±0·10

TABLE II.—*Results of Serum Flocculation Tests and Erythrocyte Sedimentation Rates in Thyroid Disease*

Clinical Group	Thymol Turbidity (Normal 0–2 units)					Thymol Flocculation (Normal 0 unit)					Cephalin-cholesterol (Normal 0)				
	0–2	3–5	>5	Total No.	% Abnormal	0	1–2	3–4	Total No.	% Abnormal	0	+ and ++	+++	Total No.	% Abnormal
Auto-immune thyroiditis	11	16	14	41	73·2	18	8	15	41	56·1	8	6	8	22	63·6
Primary hypothyroidism	42	13	0	55	23·6	48	5	2	55	12·7	26	5	1	32	18·8
Simple goitre	118	9	0	127	7·1	125	2	0	127	1·6	44	15	1	60	26·7
Thyrotoxicosis	153	13	0	166	7·8	150	12	0	162	7·4	56	31	12	99	43·4
Thyrotoxicosis with exophthalmic ophthalmoplegia	15	0	0	15	0·0	14	1	0	15	6·7	2	1	0	3	33·3
Treated thyrotoxicosis	11	3	0	16	18·8	15	1	0	16	6·3	4	2	1	8	37·5
Thyroid neoplasm	15	1	0	16	6·3	15	1	0	16	6·3	2	0	0	2	0·0

TABLE II (*continued*)

Clinical Group	Colloidal Gold (Normal 0–1 unit)					Zinc Sulphate Turbidity (Normal 5–12 units)					E.S.R. (Normal 0–12 mm./first hr.)			
	0–1	2–3	4–5	Total No.	% Abnormal	5–12	12–25	>25	Total No.	% Abnormal	Mean	Range	Total No.	% Abnormal
Auto-immune thyroiditis	13	6	21	40	67·5	5	16	7	28	82·1	23	4–78	41	68·3
Primary hypothyroidism	19	15	7	41	53·7	11	12	0	23	52·2	19	3–55	43	62·8
Simple goitre	50	33	21	104	51·9	28	15	1	44	36·4	7	1–20	106	6·7
Thyrotoxicosis	58	46	34	138	58·0	36	21	0	57	36·9	6	1–19	134	7·5
Thyrotoxicosis with exophthalmic ophthalmoplegia	7	4	3	14	50·0	6	6	0	12	50·0				
Treated thyrotoxicosis	7	6	3	16	56·3	6	2	0	8	25·0				
Thyroid neoplasm	5	4	3	12	58·3	7	3	0	10	30·0	26	5–68	15	66·7

TABLE III.—*Effect of Treatment with Thyroxine Sodium in 19 Patients with Auto-immune Thyroiditis*

	Mean Neck Circumference (in.) Mean S.D.	Thyroid Auto-antibody Tests				Serum Proteins (g. 100 ml.)			Serum Flocculation Tests (units)		E.S.R. (mm./1st hr.) Mean S.D.
		Precipitin		Complement-fixation							
		No. Pos.	% Pos.	No. Pos.	% Pos.	Albumin Mean S.D.	Globulin Mean S.D.	γ-Globulin Mean S.D.	Thymol Turbidity Mean S.D.	Thymol Flocculation Mean S.D.	
Before	14·78±1·11	16	84·2	19	100	4·23±0·40	2·66±0·67	1·41±0·57	7·0±4·5	2·0±1·5	25±15
After	13·63±1·06	13	68·4	19	100	4·59±0·36	2·34±0·62	1·00±0·39	4·0±2·2	1·0±0·1	15±9
P values	<0·001					<0·001	<0·001	<0·001	<0·001	<0·01	<0·01

The P values have been calculated from the mean difference of the values before and after treatment and its standard error and not from the differences between the means.

The thyrotoxicosis was treated by antithyroid drugs or ^{131}I therapy and tended to reverse the serum protein abnormalities; the mean serum albumin in the group of treated thyrotoxic patients being 4.7 g./100 ml. (S.D. $\pm$0.4) as compared with 4.1 g./100 ml. (S.D. $\pm$0.4) in the untreated group.

Thyroid Neoplasm

These patients had an abnormally ($P=0.001$) decreased serum albumin (mean=4.5 g./100 ml., S.D. $\pm$0.6), whereas the total serum globulins were normal. However, the α_2-globulins were increased ($P<0.05$) and the β-globulins decreased ($P<0.01$). None of the patients with thyroid neoplasm had γ-globulin levels exceeding 1 g./100 ml.

The thymol turbidity and flocculation tests and the cephalin-cholesterol were normal in most cases, although the zinc sulphate turbidity and colloidal gold reactions gave abnormal results in 30 and 58.3% of the patients respectively. The mean E.S.R. was increased (26 mm./hr.).

Discussion

An increase in the γ-globulin fraction of the serum proteins in auto-immune thyroiditis has been reported by other workers, and we have compared statistically the levels found with those in other thyroid diseases. When systemic disorders associated with serum protein abnormalities are excluded, the finding of an elevated γ-globulin—that is, exceeding 1 g./100 ml.—can be regarded as strong supporting evidence of auto-immune thyroiditis. On the other hand, a normal level of γ-globulin does not exclude the diagnosis, since in about half of our patients the γ-globulin level was below 1 g./100 ml. In patients with primary hypothyroidism we also found a slight increase in the γ-globulins, and this was particularly marked in those with precipitating antibodies to thyroglobulin. This is consistent with the current view that primary hypothyroidism and auto-immune thyroiditis share a common pathogenic basis (Goudie *et al.*, 1957; Roitt and Doniach, 1958; Buchanan *et al.*, 1958). Thus the raised globulins are presumably associated with the auto-immune response and not with hypothyroidism *per se*.

Doniach *et al.* (1960) have reported that the levels of precipitins in auto-immune thyroiditis are in the region of 700 mg./100 ml., although higher levels up to 1,890 mg./100 ml. have been reported (Mahaux and Pirart, 1959). The mean rise of γ-globulins (510 mg./100 ml.) in our patients with auto-immune thyroiditis compared with the mean value of the precipitin concentration (700 mg./100 ml.) found by Doniach *et al.* (1960) is consistent with the view that most, if not all, of the increase in the γ-globulin concentration is due to the circulating auto-antibodies. However, experimental studies (Askonas and Humphrey, 1958; Humphrey, 1960) have shown that the rise in the γ-globulins may be partly attributed to the synthesis of "non-specific" antibody.

The finding of a low serum albumin in all forms of thyroid disease eliminates this determination as a diagnostic test for auto-immune thyroiditis. The low levels found in auto-immune thyroiditis and primary hypothyroidism may be associated with diminished albumin synthesis by the liver due to occult or overt hypothyroidism (Lamberg and Gräsbeck, 1955). The striking decrease in serum albumin in thyrotoxicosis confirms previous observations (Man *et al.*, 1940; Lewis and McCullagh, 1944; Lamberg and Gräsbeck, 1955) and is probably accounted for by an increased breakdown of albumin in the peripheral tissues (Lewallen *et al.*, 1959), although impairment of hepatic synthesis has also been invoked (Lewis and McCullagh, 1944; Lamberg and Gräsbeck, 1955). The low levels of serum albumin may be a reflection of the poor intake of protein in these patients (Winzler, 1953), but the low levels in simple non-toxic goitre are not as yet explained.

Several authors have advocated the use of serum flocculation tests in the diagnosis of auto-immune thyroiditis. In the present study the cephalin-cholesterol, colloidal gold, and zinc sulphate tests were found of little practical value, since they were often positive in patients with simple goitre and thyroid neoplasm. On the other hand, the thymol turbidity and flocculation tests were found to be more specific. Although negative in about a quarter of the patients with auto-immune thyroiditis these tests were rarely positive in simple goitre and thyroid neoplasm, conditions most likely to be confused with auto-immune thyroiditis.

Finally, the value of the E.S.R. determination should not be underestimated; this was normal in 93% of patients with simple non-toxic goitre, and raised in 68% of patients with auto-immune thyroiditis.

Summary

Chemical and electrophoretic studies of the serum proteins, and a number of serum flocculation tests, and E.S.R. determinations, were carried out on a large series of patients with various thyroid diseases. The most striking findings were the raised γ-globulin level in auto-immune thyroiditis, and the decreased serum albumin level in thyrotoxicosis. A raised γ-globulin, abnormal findings in the thymol turbidity and flocculation tests, and an elevated E.S.R. are of value in the diagnosis of auto-immune thyroiditis.

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HAEMORRHAGE ASSOCIATED WITH THROMBOCYTOPENIA IN MEGALOBLASTIC ANAEMIA

BY

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Reduction in the platelet count is widely recognized as part of the dyshaemopoietic pattern in megaloblastic anaemia. The possible presentation of a megaloblastic anaemia as a haemorrhagic diathesis due to thrombocytopenia is, however, much less commonly appreciated and forms the subject-matter of this communication. Six cases were seen by us over a three-year period and three others are included by courtesy of Dr. J. L. Markson of Stobhill General Hospital, Glasgow.

Cases

The outstanding clinical features and relevant laboratory data are presented in Tables I and II, the cases being grouped according to the final diagnosis made.

Haemorrhagic Features.—All the patients were either admitted to hospital because of a haemorrhagic syndrome or developed haemorrhagic features of alarming degree shortly after admission. The main source of bleeding varied, but in each the history and clinical features also revealed evidence of a generalized haemorrhagic tendency. In Case 1 haemorrhage was from all mucosal surfaces. It was excessively severe, and was associated with a puffy pallid appearance and an initial lack of response to vitamin B_{12} and folic acid. At the time the clinical state even raised the possibility of leukaemia, and myeloid hyperplasia (described later) lent some support to this suspicion. Three cases had haematemesis and melaena (Nos. 2, 5, and 7), and in two of these there were also epistaxis and purpura (Cases 5 and 7). Uncontrollable epistaxis was associated with haematuria in Case 4, and one patient (Case 6) was admitted on separate occasions for post-partum haemorrhage and epistaxis. In Case 3 the bed was soaked with blood from the site of an intramuscular injection, and questioning elicited a history of epistaxis a few days earlier. Confluent purpura dominated in Case 9, but melaena was a further feature, and in Case 8 haematuria occurred and was accompanied by epistaxis.

Other Clinical Features.—The history indicated an insidious onset of weakness compatible with anaemia in several instances, and Cases 3 and 4 had been admitted for investigation of possible alimentary neoplasm, but it was haemorrhage in every instance which directed attention to the blood picture, and the significance of any prodromal weakness had not been fully appreciated. In Case 2 the diagnosis of pernicious anaemia had previously been made and megaloblastic anaemia as the cause of his haematemesis was quite unsuspected. The " anahaemin " he had been receiving was found to have a negligible vitamin-B_{12} content (assay by Dr. D. L. Mollin of the Postgraduate Medical School of London). In Case 8 haematuria occurred during a state of severe post-partum collapse with suppression of urine and gross electrolyte imbalance. In two cases the spleen was slightly enlarged ; in the others it was not palpable.

Blood Picture and Sternal Marrow.—Peripheral blood examination revealed varying degrees of macrocytosis and marked thrombocytopenia. The platelet count was 37,000/c.mm. or under in eight cases and was 71,000/c.mm. in the remaining case. Sternal-marrow aspiration disclosed frankly megaloblastic erythropoiesis in all cases. Erythropoiesis was also of notably immature type and sometimes was accompanied by granular-cell hyperplasia with the presence of giant myelocytes. In Case 1 the myeloid hyperplasia was excessive and indeed was questioned as leukaemic, but myeloblasts were not prominent and the megaloblastic reaction was flagrant. In two instances (Cases 4 and 8) megakaryocytes were absent ; in the others they were present and appeared normal.

Further Diagnostic Features.—Further investigations indicated several different types of megaloblastic state (Table II). Cases 1, 2, and 3 were diagnosed as Addisonian pernicious anaemia and have been maintained in full remission on intramuscular vitamin B_{12}. Addisonian pernicious anaemia was also the

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