precipitation. These three methods correlate well. The electrophoretic method was found to be the most suitable for the study of this antibody.

Antibodies to gastric mucosa were demonstrated by complement fixation and immunofluorescence. The two methods gave good correlation, the latter being more sensitive.

The antigen was localized in the cytoplasm of the parietal cells and was recovered in the particulate fraction of gastric mucosal homogenates.

The complement-fixing antibody is a 7S γ -globulin, which is specific for parietal cells and cross-reacts with gastric mucosal extracts of other species.

The parietal-cell antibody is distinct from anti-I.F.

Of patients with pernicious anaemia, 44% had auto-antibodies to I.F. The incidence of parietal-cell antibodies was 86%. This compares with 11% in controls matched for age and sex.

The immunofluorescent test may have important diagnostic applications, particularly for pernicious anaemia patients under the age of 60, where the incidence of positive reactions was 93%.

Close parallels between pernicious anaemia and auto-immune thyroiditis are drawn on the basis of clinical and immunological features.

We are grateful to Professor Sir Charles Dodds, F.R.S., for his unfailing support, and to Professor L. J. Witts for allowing one of us (K.B.T.) to use the facilities in his department. The surgeons of the Middlesex Hospital, and Mr. H. D. Johnson, of the Royal Free Hospital, kindly provided gastric tissue, and Dr. A. D. Smith some of the pernicious anaemia sera. We thank Miss Marjorie Alcock and Miss Susan Lee for their valuable assistance. This work was supported by grants from the M.R.C., the British Empire Cancer Campaign, and the Mary Kinross Charitable Fund.

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Over 60,000 students from 140 countries were studying in Britain in 1961 to 1962 according to *Overseas Students in Britain*, a new edition of which has been issued by the London Conference on Overseas Students. Just under a quarter of the total—13,293—were in universities, and of these 2,138 were reading medicine. (British Council, London Overseas Students Department, 3 Hanover Street, London W.1, price 1s. 6d.)

THYROID AUTO-ANTIBODIES IN PERNICIOUS ANAEMIA

BY

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The reported impairment of vitamin B₁₂ absorption in three out of seven patients with hypothyroidism prompted Tudhope and Wilson (1960), working in Sheffield, to investigate the incidence of pernicious anaemia in 166 patients with hypothyroidism. They found that it was surprisingly high—7.8% in the whole series and 10.3% if those with previous thyroidectomy or with thyrotoxicosis treated with radioiodine or x-irradiation were excluded. The present investigation approaches this interesting association from a different aspect—namely, the incidence of thyroid disease in pernicious anaemia.

The modern view of hypothyroidism is that "classical Hashimoto's thyroiditis and 'primary' hypothyroidism are clinical variants of the same pathological process" (Buchanan *et al.*, 1961). Thyroid biopsy is not justifiable in an investigation of this kind, and not all cases of Hashimoto-type thyroiditis suffer from hypothyroidism. Thyroid auto-antibodies in high titre are seldom found except in the serum of persons with this type of thyroiditis (Fulthorpe *et al.*, 1961). It was considered, therefore, that examination of the serum of patients with pernicious anaemia for these antibodies was the most appropriate line of inquiry to pursue.

Patients Studied

Serum was obtained from 78 patients with pernicious anaemia and from 78 controls. The former had all presented originally to one of us as untreated cases and the diagnosis was made on the basis of (1) megaloblastic bone-marrow, (2) histamine-fast achlorhydria, and (3) satisfactory response of the anaemia to vitamin B₁₂ therapy. In many cases the serum vitamin B₁₂ level was estimated and the Schilling test carried out, providing further confirmatory evidence for the diagnosis. All but six of the cases of pernicious anaemia were on maintenance therapy and attended a follow-up clinic. One, a male, was also being treated for myxoedema of long duration, and another, a female, was suffering from rheumatoid arthritis. Age and sex of the patients are shown in Table I. The ratio of females to males was approximately 2 to 1. Only four patients were under 40 years of age.

The control sera were obtained from patients with a variety of diseases, listed in Table II, who did not suffer from thyroid disease or have an enlarged thyroid gland, and who were not selected in any other way except as necessary to ensure matching with the pernicious-

TABLE I.—Age and Sex of Pernicious Anaemia Patients Studied

	20-29	30-39	40-49	50-59	60-69	70-79	80+	Total
Males	2	—	4	4	9	6	—	25
Females	—	2	6	11	15	17	2	53
Total	2	2	10	15	24	23	2	78

anaemia group for age, by decade, and by sex. Matching is essential, since thyroid auto-antibodies are more commonly present in older females. The average age of males was identical (59.1 years) in the pernicious anaemia and control series; that of females was 61.9 years in the pernicious anaemia series and 60.6 years in the control. For the purpose only of comparing the

end of that time. The end-point was taken to be 50% haemolysis. To test the specificity of positive reactions sera were tested by the same technique, using as antigen crude 1/10 saline extracts of liver and/or kidney obtained at necropsy.

Results

Twenty-six (33%) of the pernicious anaemia cases were positive by any of the three tests employed, compared with 16 (20%) of the controls. Of the six untreated cases of pernicious anaemia, the T.R.C.A. test was positive at low titre in one and the T.C.F. test was positive to a low titre in another: all tests were negative in the remainder. The details of positive results are shown in Table III, and these are summarized in Table IV.

In the T.R.C.A. test the number of positive results in males was six in the pernicious anaemia series and none

TABLE II.—Diseases in Control Patients

Diagnosis	No. of Patients	No. of T.R.C.A. Positives	No. of T.C.F. Positives
Ischaemic cardiac disease	15 (19%)	4	2
Other cardiac and vascular diseases	15 (19%)	5	—
Diabetes mellitus	9 (12%)	1	2
Various anaemias and haematological disorders	8 (10%)	1	—
Cancer and reticulosis	7 (9%)	—	—
Peptic ulcer	7 (9%)	—	—
Miscellaneous (includes 2 cases of cirrhosis of liver and 2 cases of rheumatoid arthritis accompanying other diseases)	17 (22%)	4	—
Total	78	15	4

T.R.C.A. = Tanned red-cell agglutination test for antithyroglobulin.
T.C.F. = Thyrotoxic complement fixation test.

incidence in the two groups of high-titre antibodies—that is, 1/2,500 or higher—detected by the tanned red-cell agglutination test, a further 78 matching controls were investigated. They differed from the other controls in that a larger proportion of patients with anaemia other than pernicious anaemia (20%) was included.

Serological Tests

Three tests were applied to each serum.

1. *Tanned Red-Cell Agglutination (T.R.C.A.) Test for Antithyroglobulin.*—The technique and materials used are those of Fulthorpe *et al.* (1961). One small technical difference was in the method of diluting sera. This was done with a single pipette, which was rinsed thrice in saline and dried after each dilution. By this means titres equal to those given by the use of a separate pipette for each dilution were obtained.

2. *Precipitin Test.*—This was carried out by double diffusion in agar (Ouchterlony, 1953), using as antigen a dilute solution of a crude saline extract of thyroid gland obtained at operation. Sera giving positive results were retested with comparable extracts of liver and kidney obtained at necropsy.

3. *Thyrotoxic Complement-Fixation (T.C.F.) Test.*—The technique used was less sensitive than that used by some workers—for example, Anderson *et al.* (1959)—and was comparable in sensitivity with that of Roitt and Doniach (1958). The antigen was prepared and tested as described by the latter workers. Its potency was checked several times during the investigation against stored sera of high, moderate, and low titre. No diminution in potency was detected. Sera were first screened at dilutions of 1/5 and 1/20 in physiological saline. Those giving positive results were titrated, using accurate serum dilutions of 1/5 and 1/20, and preparing from the latter by doubling dilution a range from 1/20 to 1/320. The test itself was carried out in tubes using 0.1-ml. volumes of each reagent. One volume each of diluted serum, antigen, and fresh guinea-pig complement (2 M.H.D.) were incubated for 30 minutes at 37° C. In a control tube physiological saline was substituted for antigen. After incubation one volume of a 2.5% suspension of sheep red cells sensitized by rabbit anti-sheep haemolytic serum (Burroughs Wellcome) was added to each tube. The tubes were then incubated for a further 10 minutes at 37° C. and read visually at the

TABLE III.—Details of Positive Results with Tests for Thyroid Auto-antibodies

Sex	Age	T.R.C.A. Titre	Precipitin	T.C.F. Titre
<i>Pernicious Anaemia (26)</i>				
F	71	1/25	—	—
F	54	1/25	—	—
F	50	1/2,500	+	1/40
F	54	1/25,000	+	1/320
F	51	—	—	1/5
F	64	1/2,500	—	1/40
F	70	—	—	1/40
F	49	—	—	1/10
F	75	1/250	—	1/10
F	62	—	—	1/30
F	71	1/2,500	—	—
F	54	—	—	1/30
F	70	1/250	—	1/40
F	43	1/25	—	—
F	61	—	—	1/10
F	66	1/25	—	—
F	53	—	—	1/5
F	71	1/5	—	1/20
F	49	1/25	—	—
F	75	1/25,000	+	1/160
M	70	1/25	—	1/5
M	69	1/25	—	—
M	59	1/25	—	—
M	72	1/25	—	—
M	47	1/2,500	—	1/5
M	65	1/5	—	—
Total	..	19	3	16
<i>Controls (16)</i>				
F	61	—	—	1/30
F	78	1/25	—	—
F	72	1/250	—	1/40
F	70	1/5	—	—
F	74	1/5	—	—
F	78	1/25	—	—
F	65	1/2,500	—	1/10
F	80	1/5	—	—
F	53	1/25	—	1/5
F	54	1/5	—	—
F	68	1/25	—	—
F	73	1/250	—	—
F	59	1/25	—	—
F	53	1/25	—	—
F	77	1/25	—	—
F	69	1/25	—	—
Total	..	15	—	4

TABLE IV.—Summary of Results

	Precipitin and T.C.F. Tests					T.R.C.A. Test				
	Total Tested	Positive Results				Total Tested	Positive Results			
		Precipitin		T.C.F.			All Titres		1/2,500 or Higher	
		No.	%	No.	%		No.	%	No.	%
Males:										
Pernicious	25	—	—	2	8.0	25	6	24.0	1	4.0
anaemia	25	—	—	—	—	50	1	2.0	—	—
Controls										
Females:										
Pernicious	53	3	5.7	14	26.4	53	13	24.5	5	9.4
anaemia	53	—	—	4	7.6	106	21	19.8	1	0.9
Controls										

in the 25 controls. This is a significant difference ($\chi^2=4.73$, $P<0.05$), and the same is true of females at titres of 1/2,500 or higher. At this level there were 5 positive results in 53 females with pernicious anaemia and 1 in 106 controls ($\chi^2=4.87$, $P<0.05$). In the whole pernicious anaemia group there were 6 high-titre positive results in 78 cases as compared with 1 in 156 controls, a difference which is also significant ($\chi^2=6.64$, $P<0.05$).

In the precipitin test there were in the pernicious anaemia series three positive results—one weakly positive, two strongly so—and none in the controls. This is not a statistically significant difference. No precipitin reaction was obtained with other tissue extracts. In the T.C.F. test there was a marked difference between the females with pernicious anaemia (14) and those in the control series (4) giving a positive reaction. This is statistically significant ($\chi^2=9.5$, $P<0.05$). None of the positively reacting sera fixed complement when tested with saline extracts of liver and/or kidney.

Discussion

In the T.R.C.A. test, which is more sensitive than the others used by us, there was a significant difference in the number of positive results at high titre in cases of pernicious anaemia as compared with controls. T.C.F. positive sera were not investigated exhaustively for the non-specific autoimmune complement-fixation reaction of Gajdusek (1958), but very few of our patients came within the group of connective-tissue disorders which commonly give rise to this. Caution is required in the interpretation of low titres obtained in T.C.F. and T.R.C.A. tests. In the majority of cases these probably indicate the presence of focal thyroiditis only. However, there is no certainty about this in the individual case, because 20% of cases of Hashimoto's thyroiditis have a low-titre T.C.F. antibody (1/4–1/8) or give a negative result by this test, and 31% have low-titre (1/5–1/25) or no antithyroglobulin by the T.R.C.A. test (Fulthorpe *et al.*, 1961), although in the opinion of these workers a low titre of both antithyroglobulin and T.C.F. antibody is of more significance than a low titre of either by itself. A negative result in both tests is found in only 4% of cases of Hashimoto's disease.

The three patients with pernicious anaemia who gave a positive thyroid precipitin test can reasonably be assumed to have a diffuse thyroiditis of Hashimoto type, since this is the most reliable serological test for that disease when positive. However, a positive precipitin test is obtained in only 60% of cases of Hashimoto's disease (Fulthorpe *et al.*, 1961). Therefore the three cases with positive precipitin may not be the only cases of diffuse thyroiditis in the pernicious anaemia series, particularly in view of the increased incidence of antithyroglobulin and T.C.F. antibody. The proportion of positive results in females with pernicious anaemia in the T.R.C.A. test (high titre) and in the T.C.F. test (low titre) is similar to that obtained by one of us when examining sera from a consecutive series of females with known or suspected thyroid disease—namely, 9.4% with the T.R.C.A. test and 26.4% with the T.C.F. test in pernicious anaemia cases as against 8.7% and 27.6% in the thyroid cases. A recent survey of post-mortem material by Williams and Doniach (1962) showed an increase in the incidence and severity of thyroiditis in patients suffering from pernicious anaemia.

Our results in serum tests on cases of pernicious anaemia are akin to those obtained in rheumatoid arthritis by Buchanan *et al.* (1961) when investigating the relationship between that disease and Hashimoto's disease. In 70 unselected cases of rheumatoid arthritis these authors found a significantly greater incidence of T.C.F. antibody than in a larger series of controls, while the thyroid precipitin test was positive in two cases. We conclude, therefore, that there is probably a correlation between pernicious anaemia and Hashimoto's thyroiditis similar to that reported for connective-tissue diseases and Hashimoto's thyroiditis by a number of workers, and summarized in a recent editorial (*Lancet*, 1961). Hijmans *et al.* (1961) give evidence for the view that the latter are diseases in the pathogenesis of which auto-immune mechanisms play a part. If our contention regarding the association between Hashimoto's thyroiditis and pernicious anaemia is correct, does this mean that antoimmune mechanisms play a part in the pathogenesis of pernicious anaemia?

Schwartz (1960) demonstrated antibody to intrinsic factor in the serum of some untreated cases of pernicious anaemia. Both Hashimoto's disease and pernicious anaemia are chronic diseases in the course of which functionally active tissue disappears. The natural course of Hashimoto's disease would appear to be a progression from a diffuse thyroiditis in which round-cell infiltration predominates to a state in which fibrosis and loss of acini are the most notable features. Markson and Davidson (1956) have suggested that the gastric atrophy commonly found in pernicious anaemia may be an end-result of severe atrophic gastritis. Thus the pathological processes in these two diseases may be of similar type. Hashimoto's disease occurs in the same age-group as pernicious anaemia, and Tundhope and Wilson (1960) have drawn attention to the familial incidence of both diseases. There is, nevertheless, a notable (11 to 1) preponderance of females in Hashimoto's disease which is not found in pernicious anaemia.

It may not be necessary to invoke the explanation of autoimmunity or altered immunological response. The environment created by either disease may facilitate the development of the other, or it may be that in those patients suffering from Hashimoto's disease and pernicious anaemia in combination there is an altered immunological response which is not present in the majority of cases of pernicious anaemia.

Summary

The relationship between pernicious anaemia and primary hypothyroidism was investigated by testing the sera of patients with pernicious anaemia for the presence of thyroid auto-antibodies.

In a series of 78 patients with pernicious anaemia the tanned red-cell agglutination test was positive in 24% of males and, in titres of 1/2,500 or higher, in 9.4% of females; the thyrotoxic complement-fixation test was positive in 26.4% of females. The incidence of positive tests in these patients was significantly greater than in comparable controls.

The significance of these findings and their relation to the pathogenesis of pernicious anaemia are discussed.

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ATTITUDES OF MOTHERS TO INFANT AND CHILD HEALTH AND WELFARE CLINICS*

BY

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The infant and child health and welfare services in Britain were conceived and grew in an era when poverty and ignorance were commonplace; when most women thought it natural that a considerable proportion of the babies they brought into the world should die before they were old enough to know that they were alive. Now in terms of individual experience the loss of a baby is a rarity, and the hard core of infant mortality rests with small or deformed children whose chances have been slim from the outset. It is therefore natural that searching inquiries should be made on whether expensive facilities concerned with routine medical care of babies and small children are still necessary and, if so, what shape they should take. Such inquiries could be directed towards the most efficient method of dispensing prophylactic vaccinations and inoculations, to the problems of advising on infant management, to the most efficient methods of pre-symptomatic diagnosis, and so on. The present paper, however, confines itself to a study of the attitudes of some mothers to the existing services. Mothers, after all, are not only the customers but in the last resort the people in whose hands the health of the baby lies.

Methods

The Sample.—The research originated in the work of a study group¹ of candidates for postgraduate qualifications in public health and public health nursing that under my tutorship was concerned with certain aspects of infant and child welfare which are not pursued here.² The group did, however, draw up a questionnaire which forms the basis of the present study, and which was used in interviews with mothers attending all five L.C.C. clinics in the Metropolitan Borough of Southwark and mothers attending a mobile clinic in certain villages in the rural districts of Wing and Newport Pagnell in Buckinghamshire and clinics in Ynyshir and Pen-y-Craig in the Rhondda, Glamorganshire. These areas were

chosen arbitrarily by me, but it was borne in mind that they represented widely different environmental conditions: Southwark is in the heart of London, the Buckinghamshire villages are so remote that they are served by a mobile clinic, while in the Rhondda a highly industrial community lives in semirural surroundings.

In terms of infant mortality there is also a wide range, which varies from 33/1,000 in the Rhondda and 28/1,000 in Southwark to 18/1,000 in rural Buckinghamshire. In Southwark the mothers were interviewed by members of the group; in the other two areas they were interviewed by me; and in all three areas it was made clear to the mother that the survey was not being carried out by the local authority but by an independent body. The sampling method employed was to approach every mother attending a given session of a clinic; out of the 245 mothers approached (80 in Southwark, 75 in Buckinghamshire, and 90 in the Rhondda) there were no refusals to co-operate. It is therefore reasonable to assume that the opinions expressed were representative of those of clinic attenders in the three areas, who according to data supplied by the local authorities concerned form 70–75% of the population of mothers of pre-school children.

The Questionary.—The questions were always asked in standard form, and the answers to some of them are analysed below; but information that is not relevant to the present paper was also obtained.

Results

The Mothers.—The mean age of the mothers was 28.4 years (± 5.8) and differed very little between the three areas; but regardless of their age a disproportionate number of mothers were attending with first-born children. It is not remarkable that all the mothers aged 15–19 years and 50% of those aged 20–24 were coming with their first child; but it is very striking that the proportion remained constant at about 38% from the age group 25–29 up to and including the 15 mothers who were over 40. In 1960 in England and Wales only 5% of all mothers aged 35 and over who had a child were bearing their first live baby (Ministry of Health, 1961b). When the age of the baby is considered in terms of maternal age, 78% of the mothers aged 15–19 had infants aged less than 6 months; this proportion falls to 42% among mothers aged 20–24, but there is no further fall. Social classification (Registrar-General, 1960) by the occupation of the father is given in the Chart and compared with the distribution of all males at the 1951 Census (Registrar-General, 1954, 1955). This 10-year time lag between the control and survey information is obviously highly undesirable because there has been emigration from both Southwark and the Rhondda, but a consistent pattern is shown in the three areas—namely, that there is a deficiency of social classes I and V among the clinic attenders, which proportionately is balanced by an excess from social class III.

The Children.—No appreciable difference was found between the age structures of the samples from

¹ Dr. G. D. Chetty, South Africa; Miss K. Cole, Nigeria; Dr. R. Dirican, Turkey; Dr. W. O. Fitzpatrick, R.A.M.C.; Dr. A. N. Hepburn, R.N.; Dr. J. F. Hobson, R.A.F.; Dr. C. S. Hoffman, Ghana; Dr. E. Munoz, Colombia; Dr. J. Randhawa, India; Miss M. Saville, Britain; Miss J. Scott, East Indies; Dr. J. Visvanathan, Malaya.

² The conclusions of the Study Group are given in summary in the Annual Report of the London School of Hygiene and Tropical Medicine for 1960–61, and this summary has been abstracted in the *Lancet* (1961).

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