PAPERS AND ORIGINALS

Vitamin B_{12} Status in Pregnancy among Immigrants to Britain

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Summary

Haemoglobin, serum vitamin B_{12}, and serum and red cell folate levels have been measured in 322 pregnant immigrant women in London at their first booking and in a proportion at 34 weeks of gestation and postnatally. The Indian, East-African Indian, and Pakistani and Bangladeshi patients showed significantly lower initial mean serum vitamin B_{12} levels than the European group, the levels being lower in Hindu and Sikh patients than in Moslems. The patients of West Indian, Indian, and East-African Indian origin showed significantly lower initial mean haemoglobin levels than the immigrants from European countries. Though there was no overall correlation between haemoglobin and serum vitamin B_{12} level the incidence of hypersegmented polymorphs and macrocytosis in the peripheral blood was highest in the Indian and East-African Indian patients, and both these features were particularly frequent in patients with subnormal serum vitamin B_{12} levels. Only one patient, however, had overt megaloblastic anaemia due to vitamin B_{12} deficiency. The Indian patients whose red cell folate levels were less than 200 ng/ml also had a lower mean serum vitamin B_{12} level than those with red cell folate levels greater than 200 ng/ml. The Indian patients had smaller babies than the Europeans but this was not related to the differences in vitamin B_{12} status between the two groups. However, out of 39 babies of the Indian group 5 (13%) showed subnormal serum vitamin B_{12} levels in the first 10 days of life, the lowest level being 120 pg/ml.

Though there was an overall statistically significant fall in serum vitamin B_{12} between first booking and 34 weeks of pregnancy there was no significant fall in serum vitamin B_{12} in those who initially had subnormal levels. Thus many Indian women are vitamin B_{12} deficient in pregnancy, and this is associated with morphological blood abnormalities in many cases, but megaloblastic anaemia due to this deficiency is relatively infrequent.

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Introduction

Subjects who eat no food of animal origin (vegans) are liable to develop vitamin B_{12} deficiency. By far the largest group of vegans are Indians, especially religious Hindus. Low serum vitamin B_{12} levels are common in India (Banerjee and Chatterjea, 1960) and have been noted in Indian subjects who have emigrated to Great Britain but have not appreciably changed their dietary habits (Stewart *et al.*, 1970; Elwood *et al.*, 1972). In some the deficiency causes megaloblastic anaemia (Stewart *et al.*, 1970; Britt *et al.*, 1971).

The high proportion of Indian women attending an antenatal clinic provided an opportunity to study the incidence and severity of vitamin B_{12} deficiency in Indian subjects in Britain during pregnancy and to compare this with the incidence of the deficiency in other immigrants. It was also possible to determine whether the deficiency is a significant cause of anaemia in pregnancy or affects the vitamin B_{12} status or birth weight of the infants.

Patients and Methods

A total of 322 immigrant women were selected consecutively when attending for first booking between February and July 1971 at the antenatal clinic of the West Middlesex Hospital. Some had already been given combined iron and folic acid tablets by their own doctors and it was not possible to assess reliably which of them were actually taking these tablets. Of the 322 women 239 were in the first 16 weeks of pregnancy and

only six were over 28 weeks when first seen. They were grouped according to their country of origin, as follows: (1) Central Africa (7 patients), (2) West Indies (19), (3) Far East (15), (4) Anglo-Saxon and U.S.A. (16), (5) Middle East and North Africa (11), (6) Europe (63), (7) Pakistan and Bangladesh (28), (8) India and Sri Lanka (140), and (9) East-African Indian (Kenya and Uganda Indian) (23). The mean date of booking did not differ among these groups. The subjects in groups 7, 8, and 9 were further divided into Hindus, Sikhs, and Moslems, where this was known, and the patients in groups 1-5 were grouped as "other religions." Over a third of the patients were available for repeat studies at 32-34 weeks of pregnancy. At the time of each investigation a blood film was examined for macrocytic red cells and hypersegmented polymorphs and blood was taken for haemoglobin estimation and microbiological assay. Seventy-five women from groups 7, 8, and 9 were further studied in the immediate postpartum period and a vitamin B_{12} absorption test was carried out in 18 of these patients some weeks after delivery. Serum vitamin B_{12} levels were also measured in either cord or capillary blood or both taken during the first 10 days of life in 52 infants of mothers in groups 7, 8, and 9, the capillary blood being collected at the time of the Guthrie test. The bone marrow was examined in the postpartum period in 13 Indian patients found to have low serum vitamin B_{12} levels in pregnancy.

Routine haematological methods were those described by Dacie and Lewis (1968). Hypersegmented polymorphs were considered to be present if examination of 100 polymorphs in the film showed one six-lobed cell or more than five five-lobed cells. Macrocytes were identified by light microscopy by one observer. Vitamin B_{12} was assayed by using *Euglena gracilis* z strain (normal range 160-925 pg/ml; Anderson, 1964), serum folate by using *Lactobacillus casei* ATCC 1469 (normal range 6-21 ng/ml; Waters and Mollin, 1961), and red blood cell folate by using *L. casei* (normal range 160-640 ng/ml of packed red blood cells; Hoffbrand *et al.*, 1966). Urinary methylmalonic acid was estimated in a 24-hour urine sample after an oral dose of 12 g L-valine by a thin-layer chromatography method (Dreyfus and Dubé, 1967). Vitamin B_{12} absorption was determined using an oral dose of 1 μg ^{58}Co-labelled vitamin B_{12} and a whole-body counting technique (Callender *et al.*, 1964); in our hands normal subjects absorb more than 40% of the dose.

TABLE I—*Range and Mean Haemoglobin Concentrations and Proportion of Patients with less than 11·0 g/100 ml among Patients at First Booking* (1) *and at 34 Weeks of Gestation* (2)

Country of Origin	Haemoglobin (g/100 ml) (1)			Haemoglobin (g/100 ml) (2)		
	No. of Patients	Mean ± S.D. (Range)	No. (%) < 11·0 g	No. of Patients	Mean ± S.D. (Range)	No. (%) < 11·0 g
Central Africa	7	12·7 ± 0·9 (11·0-13·5)	0	1	12·0	
West Indies	19	11·4 ± 1·1* (9·2-13·9)	6 (31·5)	8	11·3 ± 1·1 (9·4-12·6)	3 (37·5)
Far East	15	11·9 ± 0·9 (10·6-13·5)	3 (20)	3	12·2 ± 0·7 (11·4-12·8)	0
Anglo-Saxon and U.S.A.	16	12·0 ± 1·2 (10·1-14·7)	2 (12·5)	4	10·9 ± 1·1 (9·4-11·8)	1 (25)
Middle East and North Africa	11	11·9 ± 1·6 (9·2-14·9)	3 (27)	4	12·6 ± 0·9 (11·8-13·8)	0
Europe	63	12·3 ± 1·2 (10·0-16·6)	7 (11)	27	12·4 ± 1·4 (10·0-16·8)	5 (18·5)
Pakistan and Bangladesh	28	12·0 ± 1·2 (8·8-13·9)	5 (18)	13	12·4 ± 1·1 (10·4-14·0)	1 (8)
India	140	11·9 ± 1·2† (8·2-16·0)	30 (21)	57	12·4 ± 1·2 (9·8-15·3)	7 (12)
East-African Indian	23	11·5 ± 1·1* (9·1-13·3)	6 (26)	9	11·2 ± 1·5† (9·4-13·0)	4 (44)
Total	322	11·9 ± 1·2 (8·2-16·6)	62 (19)	126	12·2 ± 1·3 (9·4-16·8)	21 (17)

Significant differences from European group: *0·001 < P < 0·01; †0·01 < P < 0·05.

TABLE II—*Range and Mean Serum Vitamin B_{12} Concentrations and Proportion of Patients with less than 160 pg/ml among Patients at First Booking* (1) *and at 34 Weeks of Gestation* (2)

Country of Origin	Serum Vitamin B_{12} (pg/ml) (1)			Serum Vitamin B_{12} (pg/ml) (2)		
	No. of Patients	Mean ± S.D. (Range)	No. (%) < 160 pg	No. of Patients	Mean ± S.D. (Range)	No. (%) < 160 pg
Central Africa	7	412 ± 194* (156-696)	1 (14)	1	448	
West Indies	19	333 ± 141† (124-672)	2 (10·5)	8	304 ± 135‡ (128-440)	3 (37·5)
Far East	15	312 ± 141 (88-660)	2 (13)	4	230 ± 136 (78-408)	1 (25)
Anglo-Saxon and U.S.A.	16	286 ± 84 (142-484)	1 (6)	4	211 ± 105 (82-328)	1 (25)
Middle East and North Africa	11	273 ± 101 (80-440)	2 (18)	4	260 ± 166 (72-468)	1 (25)
Europe	62	262 ± 72 (100-436)	7 (11)	25	222 ± 66 (105-352)	6 (24)
Pakistan and Bangladesh	28	202 ± 78* (80-378)	10 (36)	11	153 ± 67† (60-237)	5 (45·5)
India	139	175 ± 99* (44-576)	76 (55)	53	142 ± 66* (36-304)	33 (62)
East-African Indian	23	175 ± 83* (72-376)	12 (52)	9	131 ± 66† (52-260)	7 (78)
Total	320	224 ± 115 (44-696)	113 (35)	119	182 ± 96 (36-468)	57 (48)

Significant differences from European group: *P < 0·001; †0·001 < P < 0·01; ‡0·01 < P < 0·05.

TABLE III—*Red Cell and Serum Folate Levels of Patients and Birth Weights of Babies*

Country of Origin	Red Cell Folate (ng/ml)		Serum Folate (ng/ml)		Birth Weight (g)	
	No. of Patients	No. (%) < 200 ng	No. of Patients	< 6·0 ng (%)	No. of Patients	Mean ± S.D.
Central Africa	7	1 (14)	7	3 (43)	3	3,070 ± 411
West Indies	19	5 (26)	19	10 (53)	13	3,238 ± 492
Far East	15	3 (20)	15	3 (20)	12	3,176 ± 497
Anglo-Saxon and U.S.A.	16	0	16	5 (31)	10	3,565 ± 556*
Middle East and North Africa	11	0	11	1 (9)	8	2,953 ± 322
Europe	62	14 (23)	63	17 (27)	46	3,252 ± 415
Pakistan and Bangladesh	27	5 (19)	28	12 (43)	21	3,090 ± 428
India	137	35 (26)	139	50 (36)	110	2,953 ± 495†
East-African Indian	22	5 (23)	23	8 (35)	18	2,842 ± 484‡
Total	316	68 (22)	321	109 (34)	241	3,067 ± 494

Significant differences from European group: *0·01 < P < 0·05; †P < 0·001; ‡0·001 < P < 0·01.

Results

The haemoglobin and serum vitamin B_{12} levels at first booking and at 34 weeks are shown in tables I and II, and the folate levels and birth weights, where known, are shown in table III.

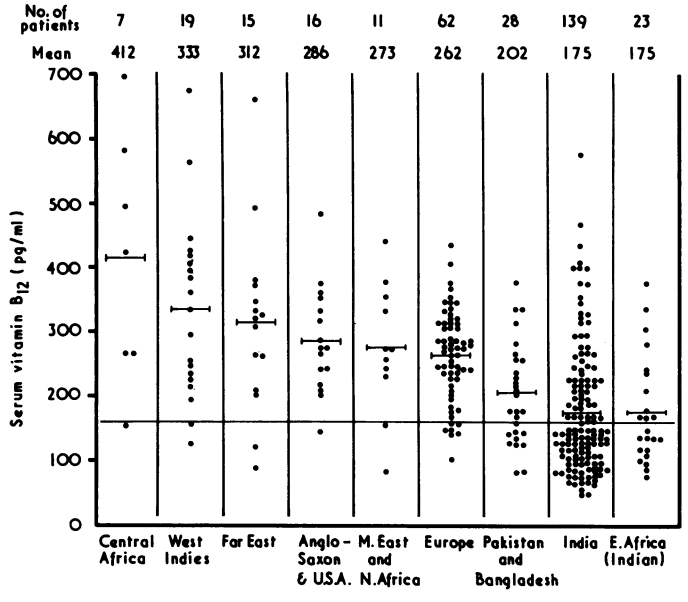
FIRST BOOKING

Haemoglobin

The mean haemoglobin in the whole group was 11·9 g/100 ml. Sixty-two of the 322 patients (19%) showed haemoglobin levels less than 11·0 g/100 ml (table I). The highest incidence of such low haemoglobin levels (31·5%) was found in the West Indian group, and the mean haemoglobin concentration in each of the West Indian, Indian, and East-African Indian groups was significantly lower than that of the European group (table I).

Serum Vitamin B_{12}

Relation to Country of Origin and Religion.—The mean serum vitamin B_{12} level of the whole group was 224 pg/ml (table II). Of the 320 patients tested 113 (35%) showed subnormal levels (<160 pg/ml), and these were most frequent in the Indian (55%), East-African Indian (52%), and Pakistani and Bangladeshi (36%) groups, who all had mean levels significantly lower than that of the Europeans (see chart). There was no difference in the mean serum vitamin B_{12} levels in the patients in these three groups (7, 8, and 9) according to the length of pregnancy at first booking. In contrast the mean serum vitamin B_{12} levels of the Central African and West Indian immigrants were significantly higher than that of the European group. Among the patients in groups 7, 8, and 9 the mean serum vitamin B_{12} levels in the Hindu, Sikh, and Moslem patients were all significantly lower than the mean serum vitamin B_{12} level of the patients of "other religions" ($P < 0.001$, $P < 0.001$, $0.01 < P < 0.05$, respectively) (table IV). The Hindu and Sikh patients had significantly lower mean serum vitamin B_{12} levels than the Moslem patients ($P < 0.001$, $0.01 < P < 0.05$, respectively). There was no significant difference, however, between the mean serum vitamin B_{12} levels of the Hindu and Sikh patients.



Serum vitamin B_{12} levels in 320 patients at first booking. Patients are divided according to country of origin and countries are arranged in descending order of mean serum vitamin B_{12} level.

TABLE IV—*Range and Mean Serum Vitamin B_{12} Level (pg/ml) at First Booking and at 34 Weeks of Gestation and Proportion of Patients with less than 160 pg/ml among Patients of Countries 7, 8, and 9 (see text) divided according to Religion (Where This was Known)*

		Hindu	Sikh	Moslem
First booking	No. of patients	20	30	14
	Range	68-404	48-404	80-332
	Mean ± S.D.	137 ± 92	155 ± 92	221 ± 74
	No. (%) < 160 pg	15 (63)	19 (63)	3 (21)
34 Weeks	No. of patients	11	12	9
	Range	66-256	44-304	60-237
	Mean ± S.D.	151 ± 69	148 ± 80	150 ± 69
	No. (%) < 160 pg	7 (64)	8 (67)	4 (44)

Relation to Age, Gravidity, Haemoglobin, and Birth Weight.—There was no difference in age, gravidity, or haemoglobin concentration between the patients with serum vitamin B_{12} levels above and below 160 pg/ml. This was true for all 320 patients and for patients in groups 7, 8, and 9 considered alone. On the other hand, among all 320 patients those with serum vitamin B_{12} levels less than 160 pg/ml had babies of significantly lower birth weight (mean 2,976 ± 526 g) than patients with normal serum vitamin B_{12} levels (mean 3,128 ± 466 g) ($0.01 < P < 0.05$). The Indian and East-African Indian patients had significantly smaller babies than the Europeans, however ($P < 0.001$ and $0.001 < P < 0.01$, respectively) (table III), and when the Indian and East-African Indian groups were considered alone there was no relation between serum vitamin B_{12} level at first booking and birth weight. It is probable, therefore, that it is only because of the large proportion of Indian immigrants in the whole group that the overall relation between low serum vitamin B_{12} and birth weight was found (the Indian immigrants having a high incidence of small babies and low serum vitamin B_{12} levels). An incidental finding was that the Anglo-Saxon and U.S.A. group had babies of significantly greater birth weight on average than the Europeans ($0.01 < P < 0.05$) (table III).

Folate

Many of the patients were already taking prophylactic folic acid before their first booking and so had greatly raised folate levels in the blood. The mean serum and red cell folate levels were therefore difficult to interpret. Of 321 patients tested 109 (34%) showed serum folate levels less than 6·0 ng/ml, and of 316 tested 24 (7·6%) showed subnormal red cell folate levels and 68 (22%) had red cell folate levels less than 200 ng/ml (table III). There was no significant difference in serum or red cell folate between immigrant groups and no overall correlation between serum folate and serum vitamin B_{12} levels. Among the Indian patients, however, those with red cell folate levels less than 200 ng/ml had a significantly lower mean serum vitamin B_{12} level (132 ± 66 pg/ml) than those with red cell folate levels greater than 200 ng/ml (190 ± 105 pg/ml) ($0.001 < P < 0.01$).

TABLE V—*Incidence of Macrocytosis and Hypersegmented Polymorphs in Peripheral Blood Films of Patients at First Booking*

Country of Origin	No. of Patients	Macrocytosis No. (%)		Hypersegmented Polymorphs No. (%)	
Central Africa	7	0		3	(43)
West Indies	19	0		5	(26)
Far East	14	0		5	(36)
Anglo-Saxon and U.S.A.	16	0		5	(31)
Middle East and North Africa	11	0		6	(55)
Europe	63	0		23	(37)
Pakistan and Bangladesh	28	0		10	(36)
India	140	9	(6·4)	80	(57)*
East-African Indian	23	2	(8·7)	14	(61)†
Total	321	11	(3·4)	151	(47)

Significant differences from European group: *$P < 0.001$; †$0.01 < P < 0.05$.

TABLE VI—*Incidence of Hypersegmented Polymorphs and Macrocytosis among Patients at First Booking and at 34 Weeks of Gestation in Relation to Serum Vitamin B_{12} and Red Cell Folate Concentrations*

	Serum Vitamin B_{12} (pg/ml)				Red Cell Folate (ng/ml)			
	First Booking		34 Weeks		First Booking		34 Weeks	
	>160 pg	<160 pg	>160 pg	<160 pg	>200 ng	<200 ng	>200 ng	<200 ng
Incidence of hypersegmented polymorphs ..	85/206 (41%)	65/113 (58%)	35/56 (63%)	26/52 (50%)	108/247 (44%)	41/68 (60%)	42/96 (44%)	7/12 (58%)
	0·001<P<0·01		N.S.		0·01<P<0·05		N.S.	
Incidence of macrocytosis	1/207 (0·5%)	10/113 (9%)	1/55 (2%)	4/52 (8%)	6/248 (2·4%)	5/68 (7%)	4/95 (4%)	1/11 (9%)
	P = 0·007		N.S.		N.S.		N.S.	

N.S. = Not significant.

TABLE VII—*Serum Vitamin B_{12} and Folate Levels and Peripheral Blood Film Appearances at First Booking and postpartum in 13 Patients whose Bone Marrows were Examined during Postpartum Period. Bone Marrow Appearances are also given*

Case No.	First Booking			Postpartum						
	Serum Vitamin B_{12} (pg/ml)	P.B.		Serum Vitamin B_{12} (pg/ml)	Serum Folate (ng/ml)	P.B.		Bone Marrow		
		H.P.S.P.	Macro.			H.P.S.P.	Macro.	Meg.	G.M.	Iron
1	48	+	0	124	6·4	0	0	0	0	+
2	51	+	+	116	3·4	+	+	++	+	+
3	64	0	0	148	4·1	+	0	+	+	+
4	68	+	0	70	—	+	0	0	+	+
5	72	+	0	156	2·8	0	0	0	0	+
6	77	+	0	220	9·0	+	0	0	+	+
7	80	+	+	164	12·0	0	0	0	0	+
8	80	+	0	104	8·2	+	0	0	+	+
9	82	0	0	132	4·1	0	0	0	0	0
10	84	+	0	88	3·1	0	0	0	0	+
11	96	+	0	124	8·2	0	0	+	+	+
12	98	0	0	184	8·6	0	0	0	0	+
13	100	+	+	184	15·2	0	0	0	0	+

P.B. = Peripheral blood. H.P.S.P. = Hypersegmented polymorphs. Macro. = Macrocytosis. Meg. = Megaloblastosis. G.M. = Giant metamyelocytes. Iron + = Iron present. Iron 0 = Iron absent.

Macrocytic Red Cells and Hypersegmented Polymorphs

Eleven patients (3·4%) showed macrocytosis and 151 (47%) hypersegmented polymorphs in the peripheral blood film (table V). All 11 patients with macrocytosis also showed hypersegmented polymorphs and were Indians from India or East Africa. Only one of the patients, however (case 2, see table VII), had obvious megaloblastic anaemia (haemoglobin 10·7 g/100 ml), confirmed by bone marrow examination in the postpartum period. The highest incidence of hypersegmented polymorphs was found in the East-African Indian (61%) and Indian (57%) groups, but in only the Indian group was the incidence significantly greater than that of the Europeans (37%) (table V). The patients with subnormal serum vitamin B_{12} levels had a significantly greater incidence of hypersegmented polymorphs (58%) than those with normal serum vitamin B_{12} levels (41%) (table VI). Considered the other way the patients with hypersegmented polymorphs showed a lower mean serum vitamin B_{12} level (200 ± 108 pg/ml) than those without this feature (243 ± 113 pg/ml) ($P < 0·001$). Of the 11 patients with macrocytosis 10 had subnormal serum vitamin B_{12} levels, and the mean level was significantly less than in those without macrocytosis (table VI). The 68 patients with red cell folate levels less than 200 ng/ml also had a just significantly greater incidence of hypersegmented polymorphs (60%) than those with red cell folate levels greater than 200 ng/ml (44%) ($0·01 < P < 0·05$) but there was no relation between lobe counts and serum folate (table VI).

32-34 WEEKS

Haemoglobin.—The mean haemoglobin concentrations of the 126 patients examined at 34 weeks was 12·2 g/100 ml (table I). The incidence of low haemoglobin levels was very similar to that found at first booking, since 21 (17%) of the 126 patients had haemoglobin levels less than 11·0 g/100 ml. Only the East-African Indian group showed a significantly lower mean haemoglobin than the Europeans ($0·01 < P < 0·05$).

Serum Vitamin B_{12}.—The mean serum vitamin B_{12} levels of 119 patients tested at 34 weeks of gestation was 182 pg/ml (table II). The mean serum vitamin B_{12} levels of the patients in each of groups 7, 8, and 9 were still significantly reduced compared with the European group ($P < 0·01$, $P < 0·001$, $P < 0·01$, respectively). The incidence of subnormal serum vitamin B_{12} levels at 34 weeks ranged from 24% in the European patients to 45% in the Pakistani and Bangladeshi patients, 62% in the Indians, and as high as 78% in the East-African Indian patients. There was a significant correlation between the first and second serum vitamin B_{12} level in the individual patients ($r = 0·74$, $P < 0·001$) with a significant overall fall in the serum vitamin B_{12} level among the 119 patients (paired t test, $t = 4·83$, $P < 0·001$). This was less marked for the Indian group alone ($0·01 < P < 0·05$), and when the patients with initial serum vitamin B_{12} levels either less than 160 pg/ml or less than 100 pg/ml at first booking were considered alone there was no significant fall in serum vitamin B_{12} level. The mean serum vitamin B_{12} level of the West Indian patients was still raised at 34 weeks compared with the Europeans ($0·01 < P < 0·05$), but too few Africans were restudied at 34 weeks to draw any conclusion about their serum vitamin B_{12} levels at that stage. In contrast to the findings at first booking, at 34 weeks there was no relation between serum vitamin B_{12} level and presence of macrocytosis or hypersegmented polymorphs in the peripheral film or red cell folate level. Nor were there any significant differences at 34 weeks between the mean serum vitamin B_{12} levels of the Hindus, Sikhs, and Moslems.

PATIENTS OF INDIAN AND PAKISTAN ORIGIN (GROUPS 7-9)

Postpartum Serum Vitamin B_{12}.—The postpartum serum vitamin B_{12} levels of 75 of these patients ranged from 19 to 860 pg/ml with a mean of 188 ± 122 pg/ml. These levels were not significantly different from those at first booking or at 34 weeks. There was a significant correlation in the individual patients between the postpartum vitamin B_{12} levels and the

vitamin B_{12} level at first booking ($r = 0.26$, $0.01 < P < 0.05$) and more closely with that at 34 weeks ($r = 0.55$, $P < 0.001$).

Postpartum Bone Marrow Changes.—Bone marrow from 13 patients who had shown lower serum vitamin B_{12} levels at first booking were examined in the postpartum period. In each of these patients the serum vitamin B_{12} level was higher in the postpartum period than at first booking (70-220 pg/ml) but in nine cases it was still below 160 pg/ml (table VII). Erythropoiesis was grossly megaloblastic in one and mildly megaloblastic in a further two; six showed giant metamyelocytes. The patient with obvious changes showed macrocytic red cells and hypersegmented polymorphs in the peripheral blood at first booking, 34 weeks, and postpartum; serum vitamin B_{12} levels at these times were 51, 120, and 116 pg/ml, and serum folate levels 8·6, 2·9, and 3·4 ng/ml, respectively.

Vitamin B_{12} Absorption.—Tests were carried out in 18 postpartum patients, all of whom had shown abnormally low serum vitamin B_{12} levels during pregnancy. In 17 vitamin B_{12} absorption was normal (43%-98%); one absorbed only 23%, corrected to 51% when repeated with added intrinsic factor.

Methylmalonic Acid Excretion.—Methylmalonic acid excretion after a L-valine load was raised in eight out of 68 patients tested in the first 10 days postpartum. Of the 60 subjects with a normal excretion 9 had serum vitamin B_{12} levels less than 100 pg/ml, 16 levels between 100 and 159 pg/ml, and 35 levels of 160 pg/ml or greater. Among the eight patients with positive test results two had serum vitamin B_{12} levels below 100 pg/ml, two had levels between 100 and 159 pg/ml, and four had levels of 160 pg/ml or higher. Thus there was no correlation between the methylmalonic acid excretion and serum vitamin B_{12} levels in these postpartum patients.

Cord and Infant Capillary Serum Vitamin B_{12} Concentrations.—In the cord blood these ranged from 30 to 848 pg/ml (mean 194 ± 152 pg/ml), and in the baby from 120 to 999 pg/ml (mean 355 ± 230 pg/ml; 16 out of 31 (52%) cord blood and 5 out of 39 (13%) baby levels were less than 160 pg/ml. The concentrations in the babies were significantly greater than in their mothers at first booking and at 34 weeks and greater than their own cord samples ($P < 0.01$ for all three). There was a significant correlation between cord and baby vitamin B_{12} levels ($r = 0.86$, $P < 0.001$). There was no correlation, however, between either the cord or baby serum vitamin B_{12} levels and the maternal serum vitamin B_{12} levels either at first booking or at 34 weeks.

Discussion

These results show a remarkably high incidence of subnormal serum vitamin B_{12} levels in pregnant Indian women who have emigrated to England from India or East Africa. Well over half of these women had subnormal levels at their first booking in pregnancy. Though a small proportion of European women also had subnormal serum levels at this early stage of pregnancy the incidence was much lower, and only one European had a level less than 100 pg/ml.

Obviously these subnormal serum vitamin B_{12} levels do not necessarily indicate severe vitamin B_{12} deficiency in every case, since only one of the patients had overt megaloblastic anaemia and none had vitamin B_{12} neuropathy. Moreover, the incidence of anaemia in the Indian group and in the whole group of 322 patients bore no relation to the serum vitamin B_{12} level. Nevertheless, there was a correlation between the serum vitamin B_{12} level and the appearances of macrocytosis and hypersegmented polymorphs in the blood at first booking, so it is likely that many of the patients had sufficient reduction of body vitamin B_{12} stores to affect bone marrow function. The poor correlation between serum vitamin B_{12} and methylmalonic acid excretion in these patients is difficult to explain, since a previous study in vegans using the same technique for methylmalonic acid estimation did show a quite good correlation (Stewart *et al.*, 1970), though it is recognized that the serum vitamin B_{12} assay is the more sensitive test (Brozovic *et al.*, 1967). It may well be that in pregnancy and the early postpartum period the interpretation of methylmalonic acid excretion as a test of body vitamin B_{12} stores is difficult. Formiminoglutamic acid excretion is known to be a poor index of body folate stores in pregnancy (Chanarin *et al.*, 1963).

The Indian patients with low serum vitamin B_{12} levels also had, on average, low red cell folate levels. Whether this was due to poor dietary intake of both vitamins or to the effects of vitamin B_{12} deficiency itself in lowering the red cell folate level or to folate deficiency lowering the serum vitamin B_{12} level is uncertain. All these factors may be involved. Hibbard and Hibbard (1972) showed that both subnormal serum vitamin B_{12} levels and megaloblastic anaemia due to folate deficiency were more common in Indians than Malays and Chinese in Singapore.

The patients with subnormal serum vitamin B_{12} levels in early pregnancy tolerated pregnancy well. It is well known that serum vitamin B_{12} levels fall during normal pregnancy and rise spontaneously in the postpartum period (Izak *et al.*, 1957; Mollin and Ross, 1957). The explanation for this fall, which was clearly shown in the present study, is thought to be preferential transfer of absorbed vitamin B_{12} to the fetus at the expense of the maternal serum vitamin B_{12} concentration. However, though the serum vitamin B_{12} levels fell significantly during pregnancy in the Indian group as a whole they were not found to fall in patients with initial subnormal levels. Moreover, by 34 weeks there was no longer any correlation between serum vitamin B_{12} level and blood film appearance, confirming that pregnancy does not appreciably aggravate subclinical vitamin B_{12} deficiency. Indeed the fetus contains only 50 μg of the vitamin, compared with normal body stores of about 3,000 μg.

The Indian patients had the smallest babies of all the patients. Cheng *et al.* (1972) found that the mean birth weight for full-term Indian infants in Singapore was lower than those for Chinese and Malay infants, which in turn were lower than the mean for British Caucasian infants. In the present study the low birth weights could not be ascribed to vitamin B_{12} deficiency, since within the Indian group there was no correlation between baby size and vitamin B_{12} level. Though the babies were apparently normal a few had subnormal serum vitamin B_{12} levels at birth. This is remarkable since newborn babies tend to have higher vitamin B_{12} levels than adults. These babies are therefore likely to develop severe vitamin B_{12} deficiency at a few months of age, particularly if they are breast-fed with milk of low vitamin B_{12} content. This has been described in India (Jadhav *et al.*, 1961) and in Western countries in infants born to mothers with unrecognized pernicious anaemia (Lampkin *et al.*, 1966). It is also possible that suboptimal vitamin B_{12} intake during gestation in humans could, by analogy with recent studies in rats, have a deleterious long-term effect on the offspring, not detected by simple weight measurement (Newberne and Young, 1973).

The cause of the low serum vitamin B_{12} levels in Indian patients is presumably dietary in nearly all cases. Thus the Sikh and Hindu patients who tend to be vegans had lower levels than Moslems from the same countries. Moreover, vitamin B_{12} absorption was normal in all but one of 18 patients tested. The Moslems did, however, have lower vitamin B_{12} levels than the European group, suggesting that their diet in many cases also provided insufficient vitamin B_{12}.

Two incidental findings are apparent from this study. Thus West Indian, Indian, and East-African Indian immigrants tend to be anaemic in pregnancy compared with Europeans. Hibbard and Hibbard (1972) showed in the mixed ethnic groups in Singapore that Indians and Malays were more anaemic in pregnancy than Chinese. West Indians and Africans were found to have higher vitamin B_{12} levels than Europeans, and this is probably because these subjects have higher levels of the vitamin B_{12} binding proteins than Europeans (Fleming, 1968).

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Effects of Long-acting Thyroid Stimulator (LATS) and LATS Protector on Human Thyroid Adenyl Cyclase Activity

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Summary

The long-acting thyroid stimulator (LATS) has been thought to be responsible for the hyperthyroidism of Graves's disease. It is detected by its effect on the mouse thyroid gland but cannot be found in all patients with hyperthyroidism. In an attempt to clarify the problem of LATS-negative hyperthyroidism, serum was obtained from untreated patients and its effect in vitro on human thyroid tissue examined, using the activation of adenyl cyclase as a measure of stimulation. Human thyroid adenyl cyclase was activated by both thyroid-stimulating hormone (TSH) and LATS. Thyroid tissue obtained from patients with Graves's disease was relatively less responsive to LATS than was non-toxic thyroid tissue. Of the 24 samples studied five contained LATS and all of these activated adenyl cyclase. The presence of LATS protector in LATS-negative hyperthyroid patients was confirmed but LATS-negative sera had no effect on human thyroid adenyl cyclase activity.

Introduction

The long-acting thyroid stimulator (LATS) is an immunoglobulin (Ig) G found in the serum of some but not all patients with Graves's disease (Adams and Purves, 1956). It has been shown experimentally to stimulate the thyroid in several species, having actions remarkably similar to those of thyroid-stimulating hormone (TSH); further, transplacental passage of LATS appears to be responsible for the development of neonatal thyrotoxicosis. For these reasons (Kriss, 1970; Munro, 1971) LATS was thought to be the cause of the hyperthyroidism in Graves's disease, and the apparent failure to find it in all patients has been attributed to inadequate sensitivity of the available bioassay methods.

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It now seems doubtful whether LATS can be incriminated as the sole cause of hyperthyroidism (McKenzie, 1972; Solomon and Chopra, 1972; Volpé *et al.*, 1972) and recently other hypotheses have been put forward to explain the problem of the LATS-negative hyperthyroid patient. The first of these postulates that, since LATS is a human IgG, there may in some cases be lack of cross reactivity with the mouse thyroid, the mouse being the species usually used for the detection of LATS. The second is based on the finding by Adams and Kennedy (1967) of LATS protector in the serum of LATS-negative hyperthyroid patients: this is an Ig, distinct from LATS, which protects LATS from neutralization by human thyroid protein. It does not bind to, or stimulate, the mouse thyroid and it has therefore been suggested that it may be a specifically human thyroid stimulator (Adams and Kennedy, 1971).

In this study an attempt has been made to explore these possibilities using human thyroid tissue. It is well established that the actions of both TSH and LATS on thyroid hormone secretion are mediated by cyclic AMP, and adenyl cyclase in the mouse thyroid was previously shown to be activated by LATS (Kendall-Taylor, 1972). This assay method for adenyl cyclase was therefore adapted for use with human thyroid tissue.

Patients and Methods

Blood Samples.—Serum was obtained from 24 patients all of whom had at the time unequivocal hyperthyroidism as judged by clinical examination, protein bound iodine estimation, and radioiodine uptake. None was receiving any drugs; though some of the patients had recurrent hyperthyroidism, none had received any treatment during the preceding year. Serum was also obtained from normal volunteers for use in control observations. Serum containing a high titre of LATS was obtained from a patient with treated hyperthyroidism and was used as a standard in all experiments. Samples were assayed for: (1) activation of adenyl cyclase in human thyroid tissue; (2) LATS activity; and (3) LATS protector. Immunoglobulin was prepared from serum by precipitation with 1·6 mol/l. ammonium sulphate and then dialysed and lyophilized. The effect of a two-fold concentrate of this material on human thyroid adenyl cyclase activity was examined and normal Ig was included in