

## Hormonal changes in thalassaemia major

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**Flynn, D. M., Fairney, A., Jackson, D., and Clayton, B. E. (1976).** *Archives of Disease in Childhood*, 51, 828. **Hormonal changes in thalassaemia major.** Patients with severe thalassaemia major suffer endocrine and other abnormalities before their eventual death from iron overload due to repeated blood transfusions. The endocrine status of 31 thalassaemic patients aged 2·5 to 23 years was investigated. Exact data were available on the rate and duration of blood transfusion in all of them and in many the liver iron concentration was also known. Although the patients were euthyroid, the mean serum thyroxine level was significantly lower, and the mean thyrotrophic hormone level significantly higher, compared with the values found in normal children. Forty oral glucose tolerance tests with simultaneous insulin levels were performed in 19 children, of whom 5 developed symptomatic diabetes and one had impaired tolerance. Previous tests on all 6 patients were available and some showed raised insulin levels possibly due to insulin resistance. 2 patients had clinical hypoparathyroidism and are described. The parathyroid hormone levels determined by radioimmunoassay in 25 patients were below the mean for the age group in all and outside the reference range in 16. Nonfasting plasma calcium levels were not reduced. Puberty was delayed in some patients. Concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) measured in urine from 7 girls and 5 boys showed considerable variation. In the boys there was an overall tendency for FSH and LH excretion to be low with regard to age, but with respect to puberty rating FSH excretions were normal or low and LH normal or raised. The girls showed a tendency for LH but not FSH excretion to be raised in relation to puberty rating. The severity of the endocrine changes was related to the degree of iron loading and is discussed in relation to previous work in which the iron loading has rarely been accurately indicated nor parathyroid status assessed.

Patients with untreated severe thalassaemia major die in early childhood from the complications of anaemia. With transfusions, life is prolonged to age 15–25 years and growth and well-being are improved. However, heavily transfused patients develop endocrine failure, hepatic cirrhosis, and later cardiac failure which is the usual cause of death. It is thought that iron overload is the major cause of these abnormalities, and it has been shown that chelating agents are of some value in diminishing both the liver iron concentration (Barry *et al.*, 1974) and the amount of hepatic fibrosis with which the iron load is associated (Risdon, Barry, and Flynn, 1975). Patients with

mild thalassaemia on the other hand may not require transfusions and may father or bear children. A few patients with thalassaemia major in a group of 31 being cared for at The Hospital for Sick Children had diabetes mellitus, hypoparathyroidism, and short stature. We were prompted therefore to look more closely at the endocrine status of this group of patients as a whole.

### Subjects and treatment

The diagnosis of thalassaemia major was established by clinical examination and haemoglobin electrophoresis. 31 patients aged 2·5 to 23 years at the time of this study had been followed as outpatients and admitted to hospital for blood transfusion every 4 to 8 weeks so as to maintain their haemoglobin concentrations between 8 and 15 g/dl. 8 of the children were receiving iron chelation therapy, consisting of desferrioxamine mesylate 0·5 g by intramuscular injection 6 days per week.

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In addition, 4 of them received 2 g diethylene-triamine penta-acetate intravenously with each unit of blood. The biochemical investigations were performed 3 weeks after the last blood transfusion.

### Methods

Complete investigations were not made on every child. Serum growth hormone was measured after Bovril stimulation (Jackson, Grant, and Clayton, 1968). Thyroid function was assessed biochemically by measuring serum thyroxine (Ryness, 1972) and thyroid stimulating hormone (Hall, Amos, and Ormston, 1971). Some patients had a glucose tolerance test and the criteria in Table I and Fig. 1 were adopted

TABLE I

Criteria for interpretation. Glucose tolerance tests

Blood glucose level mg/100 ml (mmol/l)	Clinical features of diabetes	Diagnosis
Maximum > 190 (10.5)	Present	Symptomatic diabetes
Maximum > 190 (10.5)	Absent	Chemical diabetes
Maximum < 190 (10.5) but > 120 (6.6) at 2 h	Absent	Impaired tolerance

Normal insulin response ( $\mu\text{U/ml}$ ) in glucose tolerance tests

Fasting	< 20
Peak	< 120 but > 25
At 2 h	< 30
At 2.5 h	< 20

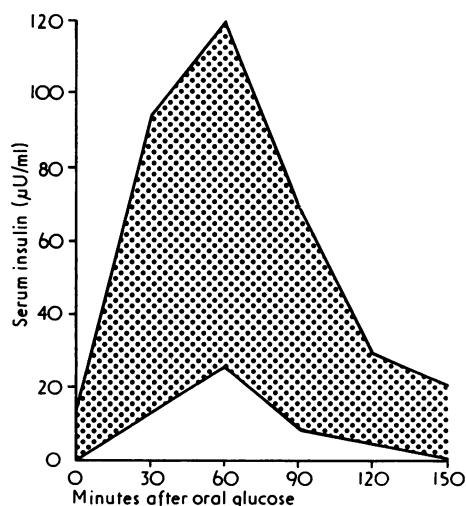


FIG. 1.—Range of insulin levels during glucose tolerance tests in control children aged 3-15 years.

for their interpretation (Grant, 1968; Milner, 1969). The children were assessed clinically for the onset of puberty and rating values assigned (Tanner, 1969). Urinary follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured by radio-immunoassay (Buckler and Clayton, 1970), with modifications.

Parathyroid function was assessed by measuring plasma total calcium using a Perkin Elmer 290B atomic absorption spectrophotometer, plasma albumin by a dye binding technique, and serum parathyroid hormone (iPTH) by radioimmunoassay (Fairney, Jackson, and Clayton, 1973). For iPTH the antiserum used was antbovine parathyroid hormone serum (guinea pig) batch AS 211/32 (supplied by the Division of Biological Standards, London); this antiserum does not have an exclusive reaction with any portion of the parathyroid hormone molecule, either at the  $\text{NH}_2$ -terminal or the  $\text{COOH}$ -terminal. The concentrations of iron in liver expressed as percentage of dry weight, determined by the method of Barry and Sherlock (1971), were taken from Barry *et al.* (1974).

### Results (Table II)

**Growth.** 5 of the patients were below the 3rd centile for height. Bovril tests performed in 3 of them produced normal responses, maximum values obtained being 19.2, 34.4, and  $> 40$   $\mu\text{IU/ml}$  serum.

**Thyroid function.** None of the children showed clinical evidence of abnormal thyroid function. However, the mean ( $\pm\text{SD}$ ) serum thyroxine of  $6.2$   $\mu\text{g}/100$  ml  $\pm 1.3$  ( $79.8 \pm 16.7$  nmol/l) was significantly lower ( $P < 0.001$ ) than the mean of  $9.5$   $\mu\text{g}/100$  ml  $\pm 1.9$  ( $122 \pm 24.5$  nmol/l) found in normal children up to 17 years old (Ryness, 1972 and unpublished observations). The mean value for serum thyroid stimulating hormone was raised ( $P < 0.001$ ), being  $3.2$   $\mu\text{U/ml} \pm 1.3$  compared with  $1.03$   $\mu\text{U/ml} \pm 0.2$  in normal children (unpublished observations).

**Carbohydrate tolerance.**—40 oral glucose tolerance tests (GTT) with insulin levels were performed in 19 children of whom 5 developed symptomatic diabetes requiring treatment with insulin and one other had impaired tolerance (Table III, Fig. 2). There was a family history of diabetes in the maternal grandmother and great grandmother of Case 13 and the father of Case 29. The insulin levels in the GTTs at the time of diagnosis of symptomatic diabetes were low in 4 patients and normal in the face of very high glucose levels in the fifth. In Case 9, the patient with impaired tolerance (blood sugar 124 mg/100 ml (6.9 mmol/l) at 120 minutes), insulin levels failed to exceed 25  $\mu\text{U/ml}$  serum at any point.

TABLE  
Details concerning

Case no.	Age (yr)*	Age at first blood transfusion (yr)	Units of blood before 5 yr†	Total units of blood†	Transfusion rate (units/yr per kg body wt in last 5 yr)	Liver iron (Fe/dry wt as %)	Chelating agent given
<i>Girls</i>							
1	4	1		25	0.794		—
2	5	3.7	13	13	0.929		—
3	7	0.4	42	76	0.615		—
4	10	0.5	52	162	0.808		—
5	10	1.6	25	108	0.868	2.537	—
6	11	2	16	132	0.526	2.180	+
7	11	1.8	32	152	0.671	2.213	+
8	12	5.6	0	89	0.543		—
9	12	0.3	64	313	1.578	5.195	—
10	13	1	30	165	0.883		—
11	16	0.3	39	236	0.477	4.097	—
12	16	1.25	51	420	0.940	3.190	+
13	18	1.4	19	250	0.629	5.0	—
14	19	1.7	27	278	0.501	3.618	—
15	23	2.4	60	435	0.467	5.080	—
<i>Boys</i>							
16	2.5	2.08	—	5	—	—	—
17	2.5	0.3	—	18	0.900	—	—
18	4.5	1.5	—	72	1.500	—	—
19	5	0.4	59	65	1.214	—	—
20	6	1	66	82	1.087	—	—
21	9.25	4.17	6	53	0.480	—	—
22	11	1.5	37	162	1.074	3.858	—
23	11.25	0.4	51	207	0.916	2.955	+
24	13	2.3	20	134	0.556	3.660	—
25	14	0.7	27	268	0.883	3.671	—
26	14	0.4	41	230	0.782	3.174	+
27	14.5	1.8	35	284	1.120	2.917	+
28	15	0.3	43	288	1.121	5.234	—
29	16	1.8	5	196	0.655	1.763	+
30	16	1.08	36	329	0.812	3.589	—
31	16	1	42	260	0.648	2.351	+

\*This is the age at which the haematological data were calculated; other results in this Table were within 18 months. †1 unit contains 200 mg iron. ‡S, splenectomized; <3, height below 3rd centile; HP, hypoparathyroidism; DA, 2nd IRP, 2nd International Reference Preparation. Conversion: Traditional units to SI—Thyroxine: 1 µg/100 ml=

TABLE III  
Glucose tolerance tests (GTT) with ages, units transfused, and liver iron concentrations

Case no.	1st GTT				2nd GTT			
	Age (yr)	Units transfused	Liver iron (% dry wt)	Result	Age (yr)	Units transfused	Liver iron (% dry wt)	Result
15	22.0	400	ND	Chemical diabetes	23.0	435	5.08*	Symptomatic diabetes
7	14.3	156	3.6 at 11 yr	„	15.9	194	5.0†	„
29	11.7	113	3.1 at 9 yr	Impaired tolerance	12.2	130	1.8 at 13 yr	„
31	11.7	166	2.8 at 10 yr	NAD	12.7	190	2.4 at 16 yr	„
26	11.6	167	3.4 at 10 yr	NAD	13.6	221	3.1 at 12.5 yr	„
9	9.5	212	ND	NAD	11.0	283	5.2 at 11.0 yr	Impaired tolerance

\*At necropsy 1 month later. †At necropsy 2.1 years later. ND, not done; NAD, no abnormality detected.

Before symptomatic diabetes or impaired tolerance was diagnosed in the 6 patients, all had GTTs with insulin levels on previous occasions. Glucose levels were normal in 2 brothers, Cases 26 and 31,

I  
The patients

Clinical features‡	Plasma GH maximum response to Bovril (µU/ml)	Stage of puberty	Urinary LH (IU 2nd IRP/ 24 h)	Urinary FSH (IU 2nd IRP/ 24 h)	Serum thyroxine (µg/100 ml)	Serum thyroid stimulating hormone (µU/ml)	Serum iPTH (pg bPTH/ml)
—	—	1	—	—	—	—	60
—	—	1	—	—	—	—	230
—	—	1	—	—	7.6	3.5	45
—	—	1	—	—	6.0	2.5	75
S	—	1	1.7	0.6	4.2	2.0	50
S	—	1	1.4	0.9	5.6	3.5	—
S, <3, GT	34.4	1	14.4	2.2	—	—	140
—	—	1	9.7	0.8	6.0	3.0	100
S	—	1	—	—	5.8	2.7	190
S, Car	—	5	1.1	0.6	3.6	2.2	40
S, <3, HP, DM, Car	19.2	1	32.0	14.7	5.7	3.0	110
S	—	1	22.0	1.3	6.2	4.2	—
S <3, HP, DM, Car	—	1	8.6	0.7	4.7	3.0	—
—	—	1	—	—	—	—	190
—	—	1	—	—	7.8	1.0	80
—	—	1	—	—	8.7	2.5	110
—	—	1	—	—	—	—	—
—	—	1	—	—	5.3	2.0	60
—	—	1	—	—	7.9	7.2	50
—	—	1	—	—	—	—	95
—	—	1	—	—	—	—	100
S	—	1	1.3	0.6	5.7	2.2	120
S	—	1	—	—	7.5	4.0	40
S, DM	—	1	—	—	4.6	2.7	95
— <3	—	2	11.3	4.3	5.9	2.5	175
— <3	> 40	1	7.7	1.6	6.2	5.2	170
S, DM	—	2	9.5	0.9	—	—	130
—	> 40	3	30	2.1	7.7	7.0	40
S, DM	—	—	—	—	6.1	2.7	40

‡ of this age.  
Clinical diabetes mellitus; GT, impaired glucose tolerance, insulin response normal; Car, clinical cardiac lesion.  
2.87 nmol/l.

but the concomitant insulin levels were not. Case 31 had raised insulin levels at 150 minutes while his younger brother had raised fasting and 2-hour insulin levels in the GTT at 10 years of age, normal insulin response at age 11, and low insulin levels when symptomatic diabetes was diagnosed at age 14 (Fig. 3). In 3 other diabetics earlier GTTs were abnormal and showed chemical diabetes; insulin levels were abnormally high in 2 (Cases 13 and 29) but low in the third patient (Case 15) who was much older. The earlier GTT with insulin levels was normal in the patient (Case 9) with impaired tolerance.

In the other 13 patients without diabetes or impaired tolerance, glucose levels in GTTs were normal, but in some concomitant insulin levels were raised. In 2 children, Cases 12 and 30, fasting insulin levels of 20 µU/ml serum were recorded on two occasions in each patient, and in 5 patients

(Cases 5, 6, 14, 22, 30) insulin levels were over 30 µU/ml at 120 minutes. One patient developed antibodies to insulin causing spuriously high insulin values during three GTTs; this patient had never received insulin therapeutically, in investigations or accidentally, so far as is known, and none of the other patients in this study had detectable levels of antibodies. In one patient, Case 28, insulin levels never exceeded 25 µU at any time during the test, but in this patient the highest blood glucose level during the GTT was 93 mg/100 ml (5.2 mmol/l).

**Parathyroid function.** 2 of the patients had clinical hypoparathyroidism. Case 15 developed tingling and carpal spasms at age 16 years after receiving 320 units of blood. The fourth metacarpals in both hands were short in her and her sister. In serum, concentrations of calcium were

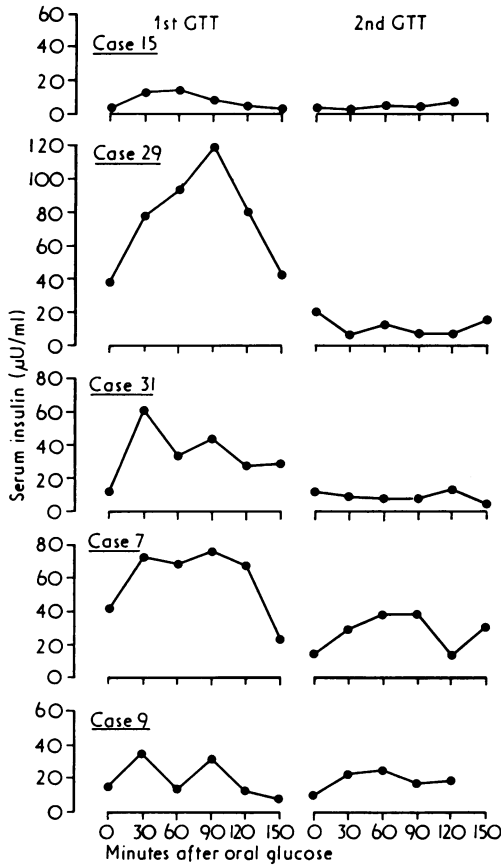


FIG. 2.—Serum insulin concentrations during glucose tolerance tests in 6 children who developed diabetes mellitus.

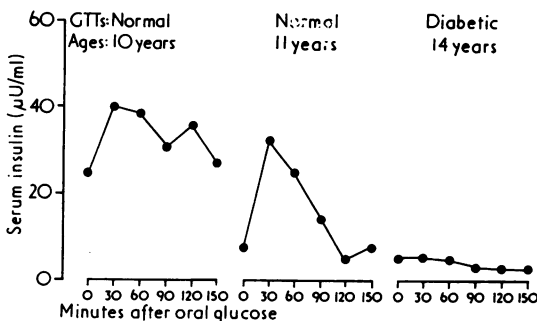


FIG. 3.—Insulin levels during serial glucose tolerance tests in Case 26.

4.5–5.0 mg/100 ml (1.13–1.25 mmol/l), phosphorus 7.6 mg/100 ml (2.5 mmol/l), alkaline phosphatase 10 KA units, and albumin 36 g/l.

Administration of parathyroid hormone increased the urinary phosphorus excretion from 4 to 43 mg/h within 5 hours, thus eliminating a diagnosis of pseudohypoparathyroidism.

She was treated with vitamin D and oral calcium and the serum calcium rose to between 7 and 8 mg/100 ml (1.8–2 mmol/l), but she continued to have occasional tingling in the hands which became a severe aching when citrated blood was transfused. Her parathyroid status remained unchanged until she died. At 22.5 years she developed chemical diabetes mellitus which became symptomatic at 23 years and she died at 23.1 years before parathyroid hormone measurements were available. Despite a careful search at necropsy the parathyroid glands could not be found.

Case 13 had short stature, absent puberty, and hepatomegaly when she developed symptomatic diabetes at 15.9 years after 207 units of blood. At age 17.3 years after 231 units she developed tingling and muscle pains with biochemical features of hypoparathyroidism though her calcium was normal at the age of 13.8 years. She was treated in the same way as Case 15 and died at 18 years.

The concentration of iPTH was measured in 25 patients and was below the mean for the age group in all of them, and outside the reference range (Clayton *et al.*, 1976) in 16 of them (Fig. 4). In spite of the low values for iPTH, concentrations of calcium in plasma were not reduced, but blood samples were collected when the subjects were in a nonfasting state.

**Gonadotrophins.** We do not possess a complete range of reference values for gonadotrophins for the whole span of adolescence. In assessing our results we have referred to our own unpublished figures on adolescents and those published by Baghdassarian *et al.* (1970) and Wide *et al.* (1973). Urinary gonadotrophins were determined in 5 boys. The results showed considerable variation, but there was an overall tendency for excretion of FSH and LH to be low with regard to age. With respect to puberty rating, values for FSH tended to be normal or low, and LH normal or raised (Table IV).

In the girls, LH and FSH excretions were normal in the 16-year old subject (Case 12) with stage 5 puberty rating. In the other 7 girls in whom LH was measured excretion was raised in 4 of them when compared with stage of puberty rather than chronological age. FSH excretion was not increased in Case 7 (aged 11) and Case 8 (aged 12), but as FSH may be undetectable in healthy children of this age it is impossible to say whether these

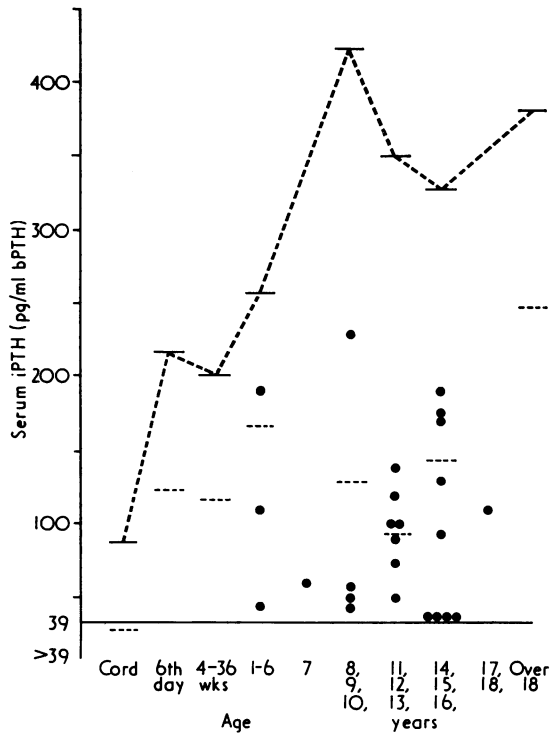


FIG. 4.—Concentrations of serum iPTH. — indicates mean for the reference range, --- indicates observed lower end of the reference range, ● indicates concentrations in the individual patients.

(Insufficient reference subjects to give a mean and range for those aged 7, 17, and 18 years.)

TABLE IV

Urinary gonadotrophin concentrations with respect to age and puberty rating in thalassaemic boys

Case no.	Relative to age		Relative to rating	
	FSH	LH	FSH	LH
24	L	L	L	N
27	N	L	N	N
28	L	L	N	H
29	L	L	L	N
30	N	N	N	H

L, lower than mean - 1 SD; N, normal; H, higher than mean + 2 SD.

values were abnormally low. In the older girls with stage 1 puberty rating, the FSH levels were low. There was therefore a disturbance of gonadotrophin excretion with a tendency for LH but not FSH to be raised in the girls in relation to the stage of puberty.

**Statistical studies.** Age at first transfusion, transfusion rate, units of blood received both up to 5 years of age and in total, content of iron in the liver, and whether or not chelating agents were given are shown in Table II. A strong positive correlation was shown by Barry *et al.* (1974) between total units of blood transfused (expressed logarithmically) and liver iron concentrations, but this is not obtainable from the data in Table II because the liver biopsies were done at various times up to 1 and 2 years before the date taken for recording the cumulative total of units transfused. Total units transfused is intended as the index of iron loading rather than liver iron concentration.

Age correlated strongly with total units transfused ( $P < 0.001$ ); age at first transfusion correlated negatively with both liver iron concentration ( $P < 0.05$ ) and total units transfused before age 5 years ( $P < 0.05$ ). The latter correlated positively with transfusion rate ( $P < 0.05$ ), confirming that more severely affected patients need early transfusion. Transfusion rate declined with age ( $P < 0.05$ ).

Plasma concentrations of thyroxine tended to fall with age ( $P < 0.05$ ) which could be a normal finding, but thyroid stimulating hormone levels did not. 5 patients were below the 3rd centile for height and this finding was significantly related to the number of units of blood received ( $P < 0.01$ ) but not to the transfusion rate.

**Discussion**

In addition to clinical diabetes mellitus, hypoparathyroidism, short stature, and delayed puberty, measurements of hormones have indicated more extensive endocrine involvement.

**Thyroxine and thyroid stimulating hormone (TSH).** The low concentrations of serum thyroxine with raised levels of serum TSH are compatible with biochemical and subclinical primary hypothyroidism. The fall in thyroxine with increasing age is compatible with subnormal function in the thyroid due to iron deposition. The contrary situation appears in the pituitary with increased TSH secretion despite iron deposition; perhaps the secretion of TSH by the anterior pituitary is more resistant to iron loading than secretion of thyroxine by the thyroid.

Abnormal thyroxine levels have not been found in thalassaemia by other workers (Zaino, Kuo, and Roginsky, 1969; Canale *et al.*, 1973; Lassman *et al.*, 1974b). The latter found the pituitary response to thyrotrophin-releasing hormone to be normal in 3 and exaggerated in 4 patients, with raised base line level in one.

**Growth hormone.** Our report of normal concentrations of growth hormone in thalassaemic patients confirms the findings of Zaino *et al.* (1969), Canale *et al.* (1973), and Toccafondi, Maioli, and Meloni (1970b). In addition, Zaino *et al.* (1969) gave growth hormone for 4 months to a thalassaemic patient who had short stature and open epiphyses but no demonstrable growth resulted. It is unlikely that growth hormone deficiency has any significant part to play in the pathogenesis of short stature in thalassaemia major.

**Gonadotrophins.** There was a tendency for LH but not FSH to be raised in both boys and girls in relation to the stage of puberty. This contrasts with the findings of Lassman *et al.* (1974b), who found low LH levels in all their post-pubertal subjects who also showed clinical hypogonadism and low concentrations of oestrogen and testosterone in the blood.

The occurrence of puberty at the normal time in Case 12, who had received 420 units of blood, is remarkable and might be considered to be due to her treatment with chelators. Even more unusual is the patient mentioned by Wolman (1964) who had received 1200 units of blood, was aged 29 and of normal height, had normal periods, and worked an 8-hour day. No mention was made of chelator therapy.

**Glucose and insulin metabolism.** Diabetes mellitus is a known but uncommon complication of thalassaemia major. Ellis, Schulman, and Smith (1954) described post-mortem studies in 4 patients with severe pancreatic fibrosis due to iron deposition but only one was diabetic during life. Engle (1964), describing primarily the cardiac involvement in thalassaemia, mentioned diabetes in 3 patients older than 20 years. Fink (1964) stated that the incidence of diabetes is lower in thalassaemia than in idiopathic haemochromatosis but did not justify this. Lassman *et al.* (1974a) claimed that only two thalassaemic patients with diabetes had been described before their report and gave two references other than the three much earlier publications cited above. Experience suggests that there are in fact many unpublished cases.

The criteria adopted for the interpretation of the insulin responses during GTTs are based on those of 13 'healthy' children described by Grant (1968) and Milner (1969) and on unpublished work from the same laboratory. The high insulin levels seen in the patients with normal GTTs suggest the possibility of insulin resistance. The earlier GTTs with insulin levels in 4 patients who later developed

symptomatic diabetes suggest initially high, followed by ultimate failure of, islet cell secretion of insulin. The insulin levels were normal or high in all but one patient with chemical diabetes, but when symptomatic diabetes was present insulin levels were low or normal in the face of high glucose levels. The single patient (Case 15) with low levels in the earlier test was considerably older and had heavier iron loading than the others. The postulated phase of high insulin secretion may have already been succeeded by islet cell failure at the time the investigations were done.

Toccafondi *et al.* (1970a) examined the plasma insulin response to oral glucose tolerance tests in 9 patients with thalassaemia major all of whom had a transfused iron load of 10 g or greater (corresponding to about 50 units of blood) but were aged only 5–10 years and presumably had received considerably less transfused iron than the majority of the patients reported here. These workers described changes in blood glucose during the oral GTTs in their thalassaemic subjects which were within the range accepted as normal in this paper but reported significantly higher blood glucose levels than in their 10 age-matched controls.

The plasma insulin levels in their control subjects were considerably higher than in our normal controls so that direct comparison with our insulin levels is not possible. However, their thalassaemic patients had lower insulin levels than their control subjects, and the cumulative output of insulin expressed in arbitrary units was lower. In the patients reported in this paper, on the other hand, insulin levels were normal or higher than normal.

Part of the discrepancy may be related to the greater age and degree of iron loading of our patients and to the development of peripheral resistance to insulin after age 10 years but before islet cell function was severely impaired by iron deposition. Support for the development of insulin resistance in older thalassaemics is provided by Kuo, Zaino, and Roginsky (1968) who found that in only 50% of older patients did the plasma glucose fall to less than half of the fasting levels after intravenous insulin. In contrast, Toccafondi *et al.* (1970b) found that plasma glucose fell normally after intravenous insulin in thalassaemic patients aged under 10 years.

Lassman *et al.* (1974a, b) found a delayed insulin response in oral GTTs in 4 older nondiabetic thalassaemics aged 7 and 21–26 years. The peak insulin level occurred at 1–3 hours instead of at one-half hour after oral glucose loading. It is perhaps significant that one of their 2 diabetic thalassaemic patients had a family history of

diabetes. These workers also found a diminished glucagon response to intravenous alanine infusion in thalassaemic patients without a family history of diabetes and suggested that these abnormalities were due to the effect of pancreatic iron loading on the alpha and beta pancreatic islet cells.

Failure of islet cells is likely to be due to the laying down of transfused iron in the pancreas which with mesenteric lymph nodes is coloured brown at laparotomy (D. Flynn and J. A. S. Dickson, personal observations). In Case 15 at necropsy there was considerable haemosiderin deposition in interstitial pancreatic tissue, glandular epithelium, and cells in the islets of Langerhans. It is likely that progressive iron deposition in the pancreas causes failure of insulin secretion, which may happen earlier in patients with a family history of diabetes.

**Parathyroid hormone.** 2 patients had clinical hypoparathyroidism (Cases 13 and 15) secondary to iron loading, and 3 similar patients have been described (Chaptal *et al.*, 1964; Gabriele, 1971; Lassman *et al.*, 1974a). In Case 15 the parathyroid glands could not be found at necropsy and it is interesting to note a similar situation at necropsy on the hypoparathyroid patient described by Chaptal *et al.* (1964). The concentration of iPTH was low in all our patients (including Case 13) in whom it was measured. Concentrations of plasma calcium fell within the normal range but since nonfasting samples were used, small changes would not have been detected.

Low levels of iPTH were unrelated to age, units transfused, or liver iron. Recent studies of parathyroid hormone have shown that its physiology is complex. It appears that the intact hormone which is the principal secretory product of the parathyroid glands undergoes cleavage so that fragments of the hormone are responsible for most of the immunoreactive material measured in the assays (Segre *et al.*, 1974). The amount of iPTH which is measured depends not only on the actual concentrations of fragments present but also upon which circulating fragments are recognized by the particular antiserum used (Arnaud *et al.*, 1974). Thus, in our patients the low concentrations of iPTH may represent a failure to secrete the normal amount of parathyroid hormone or may indicate an abnormal cleavage.

**Pituitary and end organ involvement.** Growth hormone is known to be normal or raised in thalassaemic patients. We have shown LH and TSH levels to be raised or normal despite pituitary iron deposition, either proven at necropsy or

presumed. FSH levels are normal or low. McIntosh (1973) found high levels of adrenocorticotrophin and in this finding thalassaemic patients differ from those with haemochromatosis. He also observed a reduced response to tetracosactrin. Melanin-stimulating hormone does not appear to have been measured, but a deepening skin pigmentation which is especially marked in the mid-teens and is not due to iron is often seen. These findings suggest that peripheral organ function is more severely affected by iron deposition than is pituitary function, at least at some, though clearly not all, ages.

**Abnormalities in relation to iron load.** The youngest patient to show abnormalities of glucose and insulin metabolism was Case 26 who had a serum insulin level of 37  $\mu$ U/ml at 120 minutes in his first oral GTT. He was then aged 10 years, had received 134 units of blood, and his liver iron concentration was 3.3%. The least transfused patient to show abnormalities was Case 29 who developed chemical diabetes after 112 units at age 11.7 years. However, his positive family history makes the development of diabetes unlikely to be due to iron deposition alone. The majority of patients of similar age and transfusion status were usually relatively free of symptoms. The development of abnormalities has a very variable relation to the units of blood transfused, and to the patient's age. Case 15 died after 435 units, and at age 23 years, but Case 13 died after only 250 units at age 18 years. Both were unchelated. Case 12 was regularly attending school, had a normal puberty, and was free from diabetes, though cardiac problems were present at age 16 and after 420 units. Some of her good health may have been related to chelator therapy. Though Case 13 died after 250 units, 8 patients who had received 230–280 units were reasonably well and attending school. Liver iron concentrations exceeded 5% in 4 specimens. Two were post-mortem specimens from older girls, but in contrast the others were obtained from Cases 28 and 9, aged 14.5 and 12.8 years, who were well enough to attend school though retarded in their growth.

Clearly, much further work must be done before clinical state and prognosis can be related to the variables of age, transfusion status, iron loading, and chelator treatment. The advent of serum ferritin estimations which correlate well with liver iron content (Letsky *et al.*, 1974) will enable assessments of iron stores to be made much more conveniently and easily in future.



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