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Growth Hormone Secretion Provoked by Insulin-induced Hypoglycaemia in Children of Short Stature

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The observation of Roth, Glick, Yalow, and Berson (1963) that growth hormone secretion is stimulated in response to insulin-induced hypoglycaemia has been used to identify the hypopituitary subject (Frantz and Rabkin, 1964; Kaplan, Abrams, Bell, Conte, and Grumbach, 1965). In this study, intravenous insulin-induced hypoglycaemia has been used to investigate growth hormone secretion in a group of 36 short children, all of whom were below the 3rd centile for height. It was thought, on clinical grounds, that 7 of the children (Cases 2, 4, 5, 6, 7, 8, and 9) were suffering from hypopituitarism, 2 (Cases 33 and 34) had Turner's syndrome, and there were 6 low birthweight dwarfs: these were children who, though born at term, were below 2·5 kg. in weight and whose subsequent growth has remained retarded (Cases 35, 36, 37, 38, 40, and 41). The cause of short stature in the remaining 21 children was unknown.

Clinical and biochemical data for Cases 1-10 are included in the preceding article by Hubble (1967) and of Cases 11-14 in Table II of this article.

Methods

Human growth hormone was estimated by a modification of the radio-immunoassay method of Hartog, Gaafar, and Fraser (1964a) and Hartog, Gaafar, Meisser, and Fraser (1964b). Each sample was analysed in duplicate and standards were included with each batch of analyses. A representative standard curve is shown in Fig. 1. The standard deviation of the method, derived from duplicate analyses, was $\pm$ 1·0 μmg. in the range 0-30 μmg./ml.

Blood glucose was estimated by the automated glucose oxidase method described by Discombe (1963).

Insulin test. The patients were fasted overnight for 12 hours. A needle was inserted into the anticubital vein and a slow saline infusion was started and maintained throughout the test. A three-way tap in the saline infusion line permitted administration of the insulin and withdrawal of venous blood samples with minimum discomfort to the patient. The same route was readily available for administration of glucose had the need arisen.

After a fasting blood sample had been collected, glucagon-free crystalline insulin (0·1 unit/kg.) was given. Further blood samples were collected at 10, 20, 30, 40, 60, 80, and 100 minutes subsequently for blood glucose and plasma growth hormone.

The patients were continuously observed during the test. About two-thirds of them were noticed to sweat a little and some complained of minor discomfort over the 5-minute period from 20 to 25 minutes after insulin. In no case did serious hypoglycaemic symptoms occur.

Results

Plasma growth hormone response. Seventeen of the 21 undiagnosed patients, the 6 low birthweight dwarfs, and the 2 cases of Turner's syndrome, showed a significant rise of plasma growth hormone level during the test. In 3 of these the rise was preceded by a fall from high fasting levels.

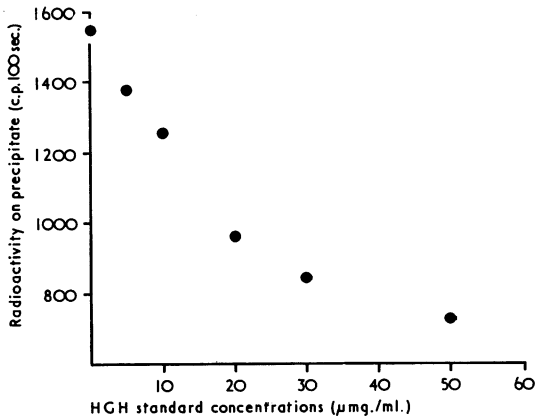

FIG. 1.—*HGH immunoassay standard curve.*

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In the 25 patients not thought to be suffering from hypopituitarism, who showed the typical growth hormone response to hypoglycaemia, the maximum levels observed in the tests ranged from 14 to 75 μmg./ml. plasma (Fig. 2, Table I). The highest growth hormone levels occurred in the youngest children, the response falling with increasing age of patient until a minimum was reached at 11-12 years. Above this age the level of growth hormone response increased again (Fig. 3).

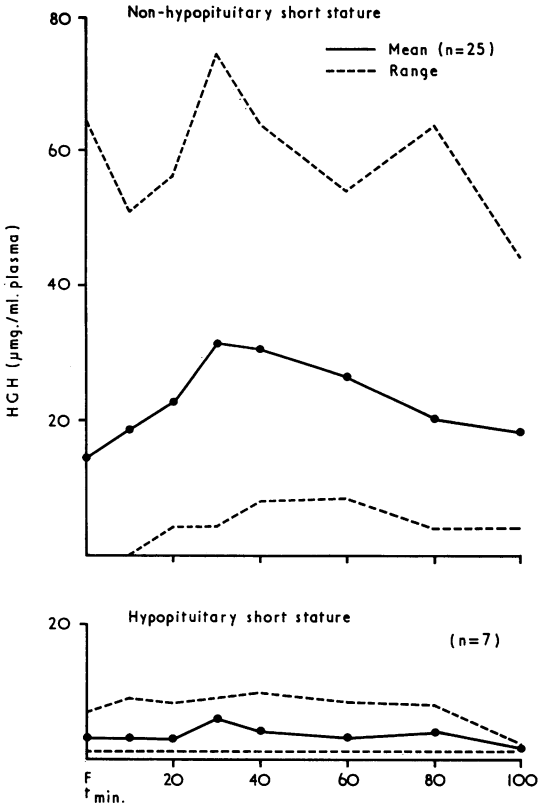

FIG. 2.—*The mean and range of plasma growth hormone concentrations in the insulin test.*

When the maximum growth hormone response was related to the expected percentage growth rate* for sex and chronological age for the normal population (Fig. 4) a highly significant correlation was obtained ($p = 0{\cdot}001$).

In the remaining 4 undiagnosed patients, summarized in Table II, the maximum growth

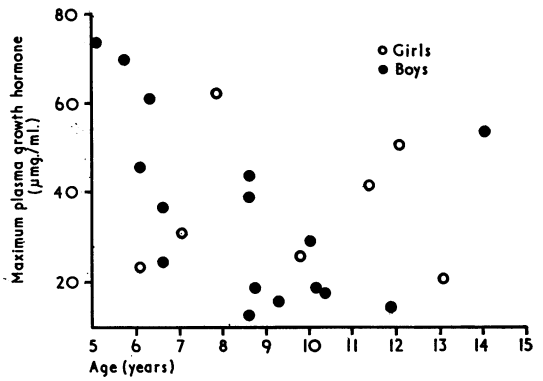

FIG. 3.—*Maximum plasma growth hormone concentrations of the non-hypopituitary children in the insulin test related to age.*

hormone concentrations following insulin-induced hypoglycaemia were less than 10 μmg./ml. (Table IIIB). Though there are no published data for insulin-induced growth hormone responses in normal children in this age range, a number of adult studies have been reported (Roth *et al.*, 1963; Hartog *et al.*, 1964a; Frantz and Rabkin, 1964). All the normal adults reported in these investigations attained a maximum growth hormone level above 10 μmg./ml. following insulin-induced hypoglycaemia.

Growth hormone levels, in the insulin tests, in the 7 hypopituitary patients were considerably lower than those in the non-hypopituitary children, the

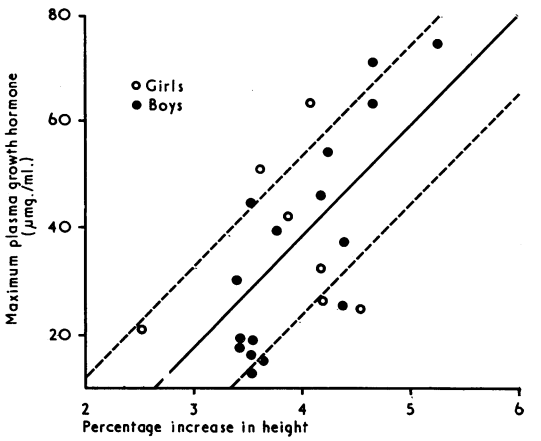

FIG. 4.—*The correlation of maximum plasma growth hormone concentrations of the non-hypopituitary children in the insulin test with the expected percentage height increment for normal children of the same age and sex.*

* The mean percentage growth rate is the ratio of growth increment for the year to the mean height for the same period expressed as a percentage. Values were calculated from the data of Tanner and Whitehouse (1959).

TABLE I

Plasma Growth Hormone (GH μmg./ml.) and Blood Glucose (mg./100 ml.) Concentrations Following Intravenous Administration of Insulin 0·1 u./kg. in Non-hypopituitary Children (maximum growth hormone response above 10 μmg./ml.)

Case No.*, Sex, and Age (yr.)				0	10	20	30	40	60	80	100 min.
15	M	7	GH	0	5	5	39	47	38	22	14
			Gluc.	62	19	5	18	28	31	32	36
16	F	8	GH	20	23	30	29	33	8	5	
			Gluc.	86	58	31	41	57	69	77	
17	M	11¼	GH	31	20	17	14	18	12	9	
			Gluc.	60	38	20	33	54	60	70	
18	M	11¼	GH	3	5	8	19	21	12	4	4
			Gluc.	64	42	19	33	52	55	59	55
19	M	10	GH	0	2	15	20	18	15	14	10
			Gluc.	80	42	13	28	39	38	39	73
20	M	9½	GH	37	38	45	43	40	25	18	12
			Gluc.	66	—	23	25	47	62	69	68
21	F	7	GH	4	4	5	10	25	17	12	
			Gluc.	65	40	17	35	53	61	61	
23	M	7½	GH	9	7	18	21	23	29	25	17
			Gluc.	53	38	20	40	37	38	45	42
24	M	7	GH	13	10	4	48	64	54	43	36
			Gluc.	62	39	36	24	49	45	52	52
25	M	9½	GH	20	18	5	—	14	14	—	13
			Gluc.	80	58	30	—	36	59	—	72
26	M	7	GH	21	32	56	71	50	42	39	—
			Gluc.	76	40	16	33	54	60	69	—
27	F	8¾	GH	63	51	47	54	49	49	64	44
			Gluc.	65	31	14	32	42	56	52	56
28†	F	10½	GH	2	14	25	27	16	20	9	
			Gluc.	79	29	16	42	42	53	81	
29	M	8¼	GH	2	0		25	26	21	25	14
			Gluc.	64	27		24	44	57	61	58
30	M	11½	GH	4	5	8	4	8	14	12	6
			Gluc.	57	37	22	38	43	49	52	54
31	M	10	GH	8	7	4	13	16	17	12	8
			Gluc.	59	42	19	35	35	44	49	52
32	M	9½	GH	1	22	34	40	35	15	6	
			Gluc.	89	52	26	32	56	59	63	
33	F	13½	GH	17	16	15	10	10	9	9	12
			Gluc.	74	57	35	32	50	60	52	56
34	F	16	GH	17	13	45	41	34	26	9	
			Gluc.	59	47	25	21	45	55	72	
35	F	14	GH	8		7	11	17	22		
			Gluc.	58		43	50	56	62		
36	M	15	GH	14	51	45	55	41	41	41	44
			Gluc.	47	38	20	27	—	28	34	34
37	M	6	GH	18	41	47	75	60	41	20	16
			Gluc.	69	44	18	27	46	56	63	68
38	F	13	GH	7	7	6	20	25	52	25	27
			Gluc.	55	40	20	18	25	37	46	49
40	F	12¼	GH	28	30	36	37	38	43	23	19
			Gluc.	71	32	17	25	41	55	66	66
41	M	8	GH	11	6	6	10	14	10	5	
			Gluc.	64	37	15	30	39	48	54	

* Cases 15-32: undiagnosed short stature; Cases 33 and 34: Turner's syndrome; and Cases 35-41: intrauterine dwarfs.

† The blood sugar concentrations in Case 28 were estimated by a modified Folin and Wu method (Harrison, 1949); a glucose oxidase method (Discombe, 1963) was used to estimate all other blood glucose levels.

TABLE II

Clinical and Biochemical Findings in 4 Patients With Poor Growth Hormone Response to Insulin-induced Hypoglycaemia but no other Evidence of Hypopituitarism

Case No., Sex, and Age (yr.)			Serum PBI (normal range 4-8 μg./100 ml.)	Serum Cholesterol (normal range 120-250 mg./100 ml.)	Urine 17-OHCS (mg./kg. 24 hr.) (Silber-Porter chromogens) (normal range 0·02-0·16)	Lateral *X*-ray View Area of Sella Turcica (sq. mm.)	Other Observations
11	M	11¾	4·8-5·3	214-216	0·10	76 (normal)	Birthweight 3·3 kg.; short stature noted throughout school life
12	F	4¼	5·4	105	0·07	28 (5th centile)	Obese (weight age 6¼ yr., height age 3¼ yr.)
13	F	8	6·6	202-254	0·03	43 (normal)	Birthweight 2·6 kg.; short stature throughout life
14	M	10¼	5·5-6·3	187-211	0·08	44 (normal)	Short stature noted at age 5

Note: All four patients were too young for assessment of secondary sexual development. None had shown evidence of hypoglycaemia at any time.

TABLE IIIA

Plasma Growth Hormone and Glucose Levels after Insulin in 7 Hypopituitary Patients

Case No., Sex, and Age (yr.)				0	10	20	30	40	60	80	100 min.
2	F	13	GH	0	0	0	—	0	0		—
			Gluc.	62	36	29	—	44	60		—
4	M	13	GH	0	0	0	1	0	0	0	0
			Gluc.	62	47	29	24	35	45	55	57
5*	M	11½	GH	2	0	3	—	2	2	2	2
			Gluc.	100	49	15	—	33	62	73	80
6	M	16	GH	3	5	4	7	3	4	6	—
			Gluc.	54	35	16	19	32	33	35	—
7	M	11	GH	7	9	7	7	8	7	8	
			Gluc.	46	33	19	26	29	33	33	
8	F	15½	GH	7	9	8	9	10	9	8	
			Gluc.	20	15	13	13	15	15	15	
9	M	17	GH	4	0	2	4	2	2	2	1
			Gluc.	60	44	12	23	38	54	60	67

TABLE IIIB

Plasma Growth Hormone and Glucose Levels after Insulin in 4 Patients of Short Stature of Unknown Cause With No Clinical Evidence of Hypopituitarism but Poor Growth Hormone Response

Case No., Sex, and Age (yr.)				0	10	20	30	40	60	80	100 min.
11*	M	11¾	GH	0	0	0	7	9	6	5	3
			Gluc.	99	56	29	39	46	62	83	88
12	F	4	GH	5	5	5	7	5	3	2	2
			Gluc.	61	45	24	33	45	44	52	54
13	F	8¼	GH	2	7	—	4	6	4	5	—
			Gluc.	61	22	—	25	27	37	39	—
14	M	10	GH	7	3	5	5	3	2	3	4
			Gluc.	61	14	18	35	39	37	51	59

In Tables IIIA and B in cases marked * blood sugars were measured by the Folin and Wu method (Harrison, 1949). A glucose oxidase method (Discombe, 1963) was used to estimate the blood glucose levels in the other cases.

initial fasting levels varying from 0-7 μmg./ml. In one patient growth hormone was completely absent throughout the test. The highest level reached in the remaining 6 was 10 μmg./ml. (Fig. 2, Table IIIA). In those hypopituitary patients who had measurable growth hormone levels there was only minimal change in growth hormone level throughout the test.

Blood glucose response. Although the blood glucose fell to hypoglycaemic levels in all 36 patients, symptoms were either slight or absent.

In the 25 patients who showed a good growth hormone response there was a sharp fall in blood glucose concentration over the first 20 minutes to approximately 30% of the fasting level (Fig. 5,

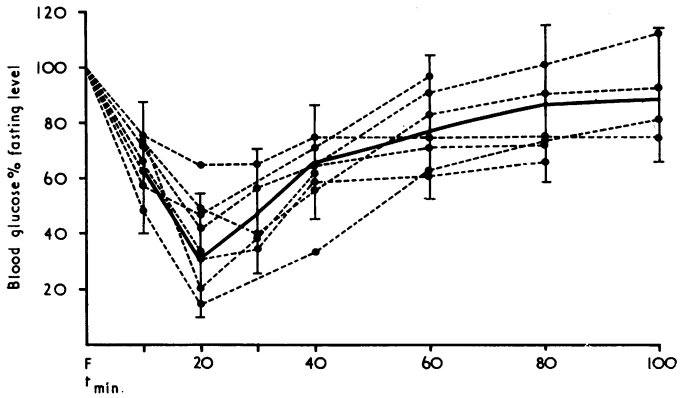

FIG. 5.—*Blood glucose changes in the hypopituitary children in the insulin test. The solid vertical bars show the mean and one standard deviation of the results in the non-hypopituitary short stature children.*

Table I). This was followed by a rapid rise over the next 20 minutes to approximately 60% of the fasting level, then a gradual rise over the next hour towards the fasting value.

In 6 of the 7 hypopituitary patients the glucose response was within the range of the non-hypopituitary group. In only 1 of the 7 was there a failure of glucose levels to return towards normal over the 100-minute period of the test (Fig. 5). This patient, though clinically symptom free, was shown to have an initial blood glucose level of only 20 mg./100 ml., which on insulin administration dropped to 12 mg./100 ml. and subsequently rose to only 15 mg./100 ml. At no time was she confused or unconscious.

The 4 patients (Cases 11, 12, 13, and 14, Table IIIB) with a poor growth hormone response, who had not previously been considered to be hypopituitary dwarfs, also had blood glucose responses to insulin which were indistinguishable from the non-hypopituitary group.

Discussion

The plasma growth hormone response to insulin-induced hypoglycaemia has not been defined for children of normal stature and growth velocity. Ethical objections preclude the investigation of the normal child by this method. The results of the insulin test reported have been assessed in the light of results of similar investigations in normal adults. An adequate response to insulin-induced hypoglycaemia has therefore been assumed to be one in which the plasma growth hormone exceeds 10 μmg./ml. plasma at some time during the test. Using this criterion all 7 children, who were already considered to be hypopituitary dwarfs on clinical grounds, failed to produce an adequate response to hypoglycaemia. Their maximum plasma growth hormone levels varied between 0-10 μmg./ml. In only 1 of the 7 was growth hormone absent throughout the test, but where growth hormone levels were measurable, very little change in concentration occurred.

Four of the undiagnosed children, not considered to be hypopituitary dwarfs, also responded with maximum plasma growth hormone levels below 10 μmg./ml. (see Table IIIB). Short stature in these children could be related to a selective deficiency of growth hormone, but, in the absence of pertinent data, the possibility of finding this apparently inadequate response in normal children cannot be excluded. In the investigation of a similar series of dwarfed children, Kaplan *et al.* (1965) also found a proportion who failed to respond adequately to insulin-induced hypoglycaemia and who had not been considered to be hypopituitary dwarfs. Poor plasma growth hormone responses to hypoglycaemia have been reported in untreated hypothyroidism (Kaplan *et al.*, 1965) and in Cushing's syndrome (Hartog *et al.*, 1964a). There was no evidence of either disorder in these 4 children.

The maximum plasma growth hormone levels in the 25 children with insulin test responses greater than 10 μmg./ml. showed a relation to age. Over the age range included in this study, the variation in growth hormone response correlated well with the percentage growth rates calculated for normal children. The progressive diminution of percentage growth rate from age 6 to 12 was accompanied by diminishing maximal growth hormone levels (Fig. 3). Similarly, over the age of 12, the increasing percentage growth rate of the pubescent growth spurt appeared to be accompanied by an increase in growth hormone response. Whether this relation of growth hormone response and rate of growth occurs in children under 6 is not known. Cornblath, Parker, Reisner, Forbes, and Daughaday (1965) gave the results of insulin tests performed on normal newborn and premature infants. Growth hormone levels up to 260 μmg./ml. were reported. Such high levels would be consistent with the very rapid growth which is a characteristic of the newborn. Cessation of linear growth at maturity, however, is not accompanied by a dramatic decrease in growth hormone production. Studies in adults indicate that the hormone continues to be produced in large amounts long after growth has ceased. It is open to question whether such an unphysiological stimulus to growth hormone secretion as insulin-induced hypoglycaemia gives a true reflection of normal pituitary function. Hunter and Greenwood (1964), in a study of random plasma growth hormone levels, showed that the highest levels were obtained in cord blood, and that there was a progressive diminution in plasma levels with age, reaching a trough in 9-10-year-old children, with a subsequent rise in the 11-17-year-old group. Random plasma growth hormone values in adults were very low. Though there was a wide scatter in each of their age-groups, their results to some extent mirror the pattern produced here by insulin-induced hypoglycaemia.

The secretion of growth hormone in the fasting individual, particularly when fasting is accompanied by the additional demands of exercise (Hunter, Fonseka, and Passmore, 1965) or by insulin-induced hypoglycaemia, is important for the maintenance of extracellular glucose concentration. Administration of growth hormone (Rabinowitz, Klassen, and Zierler, 1965) and, presumably, increased produc-

tion of endogenous growth hormone, results in the release of free fatty acids from the fat depots. This increase in circulating fatty acid provides an alternative to glucose as the primary source of energy for tissue metabolism and at the same time inhibits the metabolism of glucose (Randle, Garland, Hales, and Newsholme, 1963). Sperry (1952) has shown that cerebral tissue is unable to utilize fatty acids; and this glucose 'sparing' activity of growth hormone may be essential for normal cerebral function (Randle *et al.*, 1963). This contribution to glucose homeostasis and the conservation of protein which results from the decreased demand for glucose via the gluconeogenic pathway are important functions throughout life and would explain the continued production of the hormone in the adult.

Spontaneous hypoglycaemia is a well-recognized symptom of hypopituitarism. The prolongation of hypoglycaemia in the hypopituitary subject after intravenous insulin ('hypoglycaemia unresponsiveness'), reported by Fraser, Albright, and Smith (1941), has also been accepted as strong evidence of hypopituitarism (Prader, Illig, Széky, and Wagner, 1964; Trygstad, 1965). In 6 of our 7 clinical hypopituitary children, however, all of whom had subnormal growth hormone secretion, and the 4 undiagnosed children who also had subnormal growth hormone levels, the changes in blood glucose induced by intravenous insulin were quantitatively and qualitatively indistinguishable from the children with a good growth hormone response. Only one patient from the hypopituitary group showed 'hypoglycaemia unresponsiveness'. Similar results were found by Frantz and Rabkin (1964) in 5 hypopituitary patients, none of whom showed a delay in return of blood glucose towards fasting level after intravenous insulin. The failure to demonstrate abnormality in glucose response to insulin-induced hypoglycaemia may have been a result of partial hypopituitarism. All secreted cortisol during the insulin test, the plasma concentrations often rising to higher levels than those seen in non-hypopituitary children (B. T. Rudd and L. Stimmler, personal communication). Their unimpaired ability to secrete cortisol may enable them to compensate for the loss of growth hormone and its hypoglycaemic effect, and could account for the absence of hypoglycaemia unresponsiveness originally described in the pan-hypopituitary patient.

Summary

Insulin-induced hypoglycaemia has been used to stimulate growth hormone secretion in a group of 36 dwarfed children. All the children already diagnosed as hypopituitary dwarfs failed to produce an adequate increase in plasma growth hormone concentration. Four other children, all prepubertal, with no clinical evidence of hypopituitarism, also failed to respond normally.

In the non-hypopituitary children who responded with normal levels of growth hormone, the maximum level achieved in the test correlated with the mean percentage growth rate recorded for normal children of the same age-group.

The delayed return of blood glucose concentrations to fasting level after insulin-induced hypoglycaemia was seen in only 1 of the 11 children with inadequate growth hormone secretion.

The intravenous administration of insulin, even to the hypopituitary child, using the technique described, has been found to be a safe procedure. Only mild hypoglycaemic symptoms occurred. Cessation of the test with administration of intravenous glucose was not found necessary in any of the 36 children investigated.

We wish to thank Professor D. V. Hubble for his help and advice throughout this work and for permission to study his patients. We also acknowledge the assistance of Dr. P. Cotton, Dr. R. McArthur, and nursing staff in the insulin tests, and David Smith and Jean Fowler for expert assistance in the laboratory. The rabbit anti-human growth hormone antiserum used was a gift from Dr. Martin Hartog and the horse anti-rabbit γ-globulin antiserum was most generously supplied, without charge, by Messrs. Burroughs Wellcome.

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