THE INSULIN-GLUCOSE TOLERANCE TEST IN PITUITARY GROWTH RETARDATION

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The differential diagnosis of growth retardation has become of great importance in the past few years, since human growth hormone (HGH) treatment has become available to patients with pituitary dwarfism. The diagnosis of low birthweight dwarfism (Black, 1961) is usually easy, but before puberty the differentiation of constitutionally retarded growth (Wilkins, 1957) from pituitary dwarfism may be very difficult. The insulin tolerance test has proved, in our experience, a useful supplement to other tests in the selection of patients for HGH treatment, despite the contrary conclusions of some other workers (Colombo, Richterich, Rossi, and Feinsmith, 1962). In this paper we give our experience with the insulin-glucose test, comparing its usefulness with that of other tests of endocrine function.

Material and Methods

During the past two years the insulin tolerance test has been carried out on 22 patients between 6 and 19 years of age who were diagnosed as having pituitary growth retardation. Of these, 13 are now receiving continuous treatment with HGH; 9 have been treated for more than a year with good response, their height increments ranging from 7 to 13 cm. during the first year of treatment.

During the same period insulin tolerance tests have also been carried out on 12 patients in whom the diagnosis of constitutional growth retardation has been made, and on 15 control patients without hormonal or metabolic disorders, and of the same age distribution.

The insulin tolerance tests were carried out after the patients had been in hospital for at least three days on a moderately high carbohydrate diet. Patients with pituitary failure are known to be hypersensitive to insulin, which often causes a considerable decrease in blood sugar, followed by a very slow return to normal levels. Even when adrenaline or glucagon are injected the blood sugar rises only slowly. The usual insulin tolerance test may, therefore, be hazardous in these patients. For a standardized test without reduction of the insulin dose when insulin hypersensitivity is suspected, we have preferred the less dangerous insulin-glucose tolerance test (Engel and Scott, 1950).

Crystalline glucagon-free insulin, 0.1 I.U./kg. body weight is given intravenously. The insulin is diluted 1 : 10 in saline. 30 minutes after the insulin injection or when the first significant symptoms of hypoglycaemia appear, whichever is the earlier, the child is given oral glucose 1.75 g./kg. (in apple juice). Capillary blood for sugar determination was obtained in this series at 0, 20, 30, 45, 60, 90, 120, 150, and 210 minutes after the insulin injection, but as no significant differences between the groups were found after the administration of glucose, the test may be ended at 60 minutes. Blood sugar determinations were performed by the method of Hagedorn, Halström, and Jensen (1935).

During the first 30 minutes the child is observed closely for signs of hypoglycaemia: if these occur a blood specimen must be obtained and glucose given immediately, intravenously in severe cases. In our series of patients with pituitary dwarfism, we observed significant hypoglycaemic signs in one patient (A.S.); oral glucose was given at 25 minutes and the signs rapidly subsided.

The insulin-glucose tolerance test is less time consuming than the ordinary insulin tolerance test, where the child has to be observed closely for hypoglycaemic signs for at least an hour after insulin injection. Furthermore, with the ordinary dosage, severe hypoglycaemia often occurs in patients with pituitary hypofunction, so that the test may have to be performed more than once, after starting with one-third the usual dose.

Results

Table 1 shows the mean results of the insulin-glucose tolerance tests in the groups described. Table 2 gives further details about the tests performed on the patients with pituitary dwarfism in whom the diagnosis had been proved by response to HGH treatment over a period of more than a year.

In the normal child the blood sugar drops to about 50% of the initial fasting value in 15 to 20 minutes (Colombo *et al.*, 1962). A fall below 50% is usually said to indicate an abnormal insulin tolerance (Williams, 1962), but this is not in accord with our experience. Table 3 presents the numbers of patients in whom the test was 'abnormal' by this

TABLE 1
RESULTS OF INSULIN-GLUCOSE TOLERANCE TESTS

	No.	Blood Sugar Concentrations After (minutes) Mean $\pm$ SD									Maximum Decrease	
		0	20	30	45	60	90	120	150	210	%	SD
Controls	15	94 $\pm$ 9	45 $\pm$ 6	60 $\pm$ 13	114	142	168	157	138	91	52	$\pm$ 6.5
Constitutional dwarfism	12	92 $\pm$ 16	41 $\pm$ 11	55 $\pm$ 13	102	131	157	147	117	91	55	$\pm$ 7.5
Pituitary dwarfism, HGH treated more than 1 year	9	88 $\pm$ 15	53 $\pm$ 14	42 $\pm$ 11	94	127	149	136	118	91	52	$\pm$ 15.5
Pituitary dwarfism ?	13	93 $\pm$ 15	55 $\pm$ 14	45 $\pm$ 12	97	120	153	155	146	111	53	$\pm$ 9.5

TABLE 2
RESULTS OF INSULIN-GLUCOSE TOLERANCE TESTS IN 9 PATIENTS SHOWING GOOD RESPONSE
TO TREATMENT WITH HGH FOR MORE THAN A YEAR

Patient	Age (yr.)	Height Increment		Blood Sugar Concentration After (minutes)								Maximum Decrease (%)	
		Before Treatment (cm./yr.)	On Treatment (cm./mth.)	0	20	30	45	60	90	120	150		210
A.S.	12½	9/5	24/36	90	48	25*	107	136	210	154	141	106	72
C.S.	9½	6/4½	28/36	115	58	31	94	151	155	206	131	76	73
A.S.F.	9	23/6	16/27	95	44	38	100	140	164	125	86	94	60
T.V.	16	25/9	14/24	78	57	44	86	117	130	107	101	105	44
T.H.S.	12½	15/7½	11/24	57	47	42	63	115	129	132	154	80	26
T.R.M.	8½	11/5½	9/15	76	32	46	72	76	72	68	105	86	58
R.E.	19	28/9½	9/13	93	72	47	97	127	193	171	150	101	49
K.V.	6	6/1½	7/12	97	80	67	107	154	125	117	82	78	31
O.L.	10	16/4	7/12	90	41	39	117	123	173	140	109	97	56
Mean: ± SD	11½	2.65/1	7½/12	88 15.2	53 14.4	42 11.1	94 16.2	127 21.9	149 37.7	136 37.0	118 25.4	91 11.5	52 15.5

* 25-minute blood sample (hypoglycaemic symptoms).

TABLE 3
MAGNITUDE OF BLOOD SUGAR DECREASE DURING
INSULIN-GLUCOSE TOLERANCE TEST

	Blood Sugar Decrease of	
	> 50% (No.)	> 60% (No.)
Controls	9/15	2/15
Constitutional dwarfism	9/12	4/12
Pituitary dwarfism, HGH treated	5/9	2/9
Pituitary dwarfism ?	8/13	3/13

TABLE 4
NUMBER OF CASES SHOWING DECREASE IN BLOOD
SUGAR BETWEEN 20 AND 30 MINUTES

	No.
Controls	0/15
Constitutional dwarfism	0/12
Pituitary dwarfism, HGH treated	8/9
Pituitary dwarfism ?	12/13

criterion, showing that there was no difference between the groups. 6 patients in whom pituitary function was presumed to be normal showed a fall in blood sugar to below 40% of the initial fasting value.

During the first 20 minutes the decrease in blood sugar concentration after insulin injection seemed to be slightly greater in children with normal pituitary function than in those with hypopituitarism. The difference, however, is not statistically significant (p between 0.05 and 0.025).

Table 4 and the Figure show the numbers of patients in each group where the lowest blood sugar value was recorded not in the 20-minute but in the 30-minute sample. None of the controls or the patients with constitutional growth retardation had lower blood sugar values in the 30-minute than in

the 20-minute sample. Two of the controls had the same concentration in the 20- and 30-minute samples, perhaps because the lowest level was reached between the two blood samples.

The change in blood sugar concentration between the 20- and the 30-minute sample is significantly different when compared with the controls in both groups of pituitary dwarfism ($p < 0.001$). The group with constitutional dwarfism shows no difference from the controls ($p = 0.9-0.8$).

Only two patients originally diagnosed as pituitary dwarfs had an increase in blood sugar from the 20-minute sample to the 30-minute sample. Both these patients had normal metyrapone tests, and there may be some doubt about the diagnosis. One of them (T.R.M.) was born with a grave foetal

FIG.—Change in blood sugar concentration between 20 and 30 minutes after insulin injection in four groups of cases: proven pituitary dwarfism, unproven pituitary dwarfism, normal controls, and constitutional dwarfism. Broken lines indicate the two suspected pituitary dwarfs with normal insulin-glucose tolerance tests (see text). The blood-sugar concentrations are calculated after adjusting the fasting level to 100 mg./100 ml.

asphyxia. He may possibly have a low birth weight dwarfism, because he and a younger sister had an intrauterine growth retardation, though he has grown 9 cm. in 17 months on HGH treatment, having grown only 9 cm. in the course of the previous 42 months. The other (K.S.) may also be suffering from constitutionally retarded growth and adolescent development, but because of his extreme shortness (137 cm. at 17 years) the effect of HGH was tried. However, the response has not been very striking after 8 months of treatment (height increment 4 cm.).

We, therefore, regard the insulin-glucose tolerance test as abnormal when the blood sugar curve shows a continuous decrease until glucose is given.

Discussion

In childhood the differentiation of pituitary dwarfism from constitutional growth retardation is difficult, the diagnosis resting upon a number of features, none of which are pathognomonic.

The early diagnosis of pituitary dwarfism is important now that HGH treatment can be offered. In untreated cases our experience is the same as Kaplan's (1964), that the bone age of the pituitary dwarfed child increases with time more than does height age, so that the prognosis concerning the final height likely to be achieved with HGH therapy deteriorates with time.

The previous view (Daughaday, 1962) that pituitary growth retardation generally starts after the second year of life, is usually not familial, and is predominantly seen in boys, does not accord with our experience (Trygstad and Seip, 1964), and we regard these features as of little diagnostic value. Immunological methods for plasma HGH determinations do not as yet seem to be decisive in the diagnosis of pituitary dwarfism. Large and rapid variations in plasma HGH concentration occur during the day (Hunter, and Greenwood, 1964) and the values found in normal controls and in hypopituitarism overlap (Utiger, Parker, and Daughaday, 1962). Plasma HGH determinations after insulin hypoglycaemia may prove to be a more useful test.

The activity of the different pituitary hormones should be evaluated by the available tests. Though most pituitary dwarfs do not mature sexually, this is not always true (Antonin, 1961). The corticotrophin and thyrotrophin reserves may also be normal, so that the possibility of an isolated HGH deficiency must be considered.

Table 5 shows the main criteria on which we base the differential diagnosis between constitutional and pituitary growth retardation.

Table 6 shows the results in two other tests we have studied. The urinary excretion of 17-ketogenic steroids (17-KGS) has proved of no diagnostic value in our patients, all four groups having the same mean daily excretion of 17-KGS.

The metyrapone test, however, provides good evidence of the pituitary corticotrophin reserve. Metyrapone, 70-100 mg./kg. body weight, is given orally in 6-10 doses during 24 hours. This substance blocks cortisol synthesis, and the blood concentration of its precursor, 11- β hydroxycortisol (compound 'S'), therefore increases. With a normal pituitary, therefore, there is increased corticotrophin secretion and increased compound 'S' formation. The increase in urinary excretion of compound 'S' (THS) after metyrapone is interpreted as normal when it exceeds 1 mg./24 hr. and the increase of urinary 17-KGS excretion exceeds 100%.

The metyrapone test has been carried out in 18 patients provisionally diagnosed as pituitary dwarfism. Of these, 12 showed an abnormal and 6 a normal response. One of the latter had a craniopharyngioma

with growth retardation during the past four years, and a height increment of only 2 cm. during the past two years. In this patient the insulin-glucose tolerance test was abnormal, indicating a pituitary failure. Of the patients in Table 2 T.R.M. and R.E. had normal tests, A.S. had 300%, and T.V. 100% increase in urinary 17-KGS excretion.

None of our pituitary dwarfs have had hypoglycaemic episodes, and there are no significant differences in the fasting blood sugar levels between the groups (Table 1).

Hypoglycaemia induced by insulin is probably chiefly due to the increased peripheral uptake of glucose. The return of blood glucose concentration to normal levels may depend on several factors: (i) increased glucose release by the liver, (ii) decreased sensitivity of the peripheral tissue to insulin, e.g. caused by the increased growth hormone secretion evoked by the hypoglycaemia, (iii) an increase in the level of insulin inhibitors or antagonists, and (iv) gradual inactivation of the injected insulin.

The hypersensitivity to insulin in hypopituitarism and secondary adrenal insufficiency may be caused by changes in some or all of these factors, but we do not know which factor is most important.

Recently it has been demonstrated that insulin-induced hypoglycaemia will produce an abrupt rise in the level of HGH in plasma (Hunter and Greenwood, 1964; Roth, Glick, Yalow, and Berson, 1964). Insulin and growth hormone seem to interact and behave like a new hormone that does not stimulate either glucose or free fatty acid uptake by the peripheral tissues (Zierler and Rabinowitz, 1963). Luft and Cerasi (1964) suggest that the biological activity of circulating insulin may be masked by increased amounts of an insulin inhibitor elicited by the growth hormone.

A number of years ago Young (1938) demonstrated that injections of anterior pituitary extracts in rabbits made the animals more resistant to the hypoglycaemic effect of insulin. Our own unpublished observations demonstrate that HGH treatment of pituitary dwarfs tends to restore the insulin-glucose tolerance test to normal. The insulin tolerance test is generally considered to be a test of corticotrophin—adrenal reserve, but it may perhaps be a more specific test for the somatotrophic function of the pituitary.

Summary

The insulin-glucose tolerance test was performed on 22 patients diagnosed as pituitary dwarfism: in 9 of them the diagnosis had been confirmed by a growth response to human growth hormone. Only 2 patients had a normal test, and the diagnosis was doubtful in these.

TABLE 5
DIFFERENTIATION OF CONSTITUTIONAL FROM
PITUITARY GROWTH RETARDATION

Diagnostic signs	Constitutional Dwarfism	Pituitary Dwarfism
Familial occurrence ..	Often	At times
Growth retardation since birth ..	At times	At times
Growth velocity after third year ..	3-5 cm./yr.	1-4 cm./yr.
Puberty ..	Late	Usually absent
Bone age retardation ..	Moderate	Severe
Serum P. inorganic ..	Normal	Often low
Serum total cholesterol ..	Normal	Often high
Serum protein-bound iodine ..	Normal	Often low
Metyrapone test ..	Normal	Often abnormal
Insulin-glucose tolerance test ..	Normal	Often abnormal

TABLE 6
URINARY EXCRETION OF 17-KETOGENIC STEROIDS
AND RESULTS OF METYRAPONE TEST

	Mean Age* (yr.)	Mean Daily Excretion of 17-KGS in Urine (mg.)	Ab-normal Metyrapone Response
Normals	10.2 (6-14)	2.9	0/9
Constitutional growth retardation	9.9 (6-18)	3.3	0/12
Pituitary dwarfism, HGH treated	11.5 (6-19)	3.0	7/9
Pituitary dwarfism ?	10.6 (6-18)	3.0	5/9

* The four groups were matched for age, except that all 9 children in the normal control group, being pre-pubertal, were less than 14 years.

The test was performed on 12 patients diagnosed as constitutional growth retardation and on 15 normal controls with the same age distribution as the pituitary dwarfs; all 27 had a normal test.

In interpreting the insulin-glucose test, the decrease in blood sugar between 20 and 30 minutes best differentiated the group of pituitary dwarfs.

In this study the insulin-glucose tolerance test proved a better diagnostic aid than the metyrapone test.

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