Glucagon test for insulinoma: a chemical study in 25 cases

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SYNOPSIS The capillary blood glucose response to 1 mg of intramuscular glucagon was determined in 13 patients with insulinoma and in 33 normal controls; the insulinoma patients showed a normal initial rise, but this was followed by an abnormally large fall, reaching hypoglycaemic levels between 90 and 180 minutes in every case. In 14 insulinoma patients the response of venous blood glucose and also plasma insulin to 1 mg of intravenous glucagon was compared with 10 normal controls; there was an abnormally large rise of plasma insulin in 10 of the 14 patients, and in the majority the venous blood glucose was below normal throughout the test. In these 14 patients the plasma insulin response was also determined after oral and intravenous glucose, after oral leucine, and after intravenous tolbutamide, and the value of these tests in the recognition and differential diagnosis of insulinoma was compared with that of the intravenous glucagon test.

The diagnosis of insulinoma is often difficult. It depends in the first instance upon the recognition of hypoglycaemia as the cause of symptoms; secondly upon establishing its aetiology. Since it is impracticable to admit every patient with a suggestive history and keep him in hospital until a spontaneous episode occurs, provocative tests are frequently employed. In the past, the oral glucose test was commonly used, but its unreliability as a diagnostic procedure for insulinoma is now well recognized (Marks and Rose, 1965). The prolonged fast test though reliable is tedious, unpleasant, and expensive because admission to hospital is necessary. The intravenous tolbutamide test though valuable in the diagnosis of insulinoma (Fajans, Schneider, Schteingart, and Conn, 1961; Marrack, Rose, and Marks, 1961) does not reveal every case and is occasionally dangerous and less specific than previously thought.

With the recent demonstration by Samols, Marri, and Marks (1965a) that glucagon is a direct stimulus to insulin secretion, a new tool for the investigation of β -islet cell function became available. This paper describes a glucagon test used by us for the recognition and differential diagnosis of spontaneous hypoglycaemia in 25 cases of insulinoma and in patients with spontaneous hypoglycaemia from diverse other causes.

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MATERIALS AND METHODS

Twenty-five patients with hyperinsulinism were studied, two of them (A.B. and W.K.) on more than one occasion. In 24 patients an insulinoma was found at operation; 18 were benign and five were malignant with metastases. The remaining patient (W.K.), who at first refused operation, was operated upon four years later but no tumour found though clinically the diagnosis was not in doubt; both fasting hypoglycaemia and hyperinsulinaemia persisted postoperatively. In addition two patients with hypoglycaemia due to extrapancreatic neoplasms, four with hepatogenous hypoglycaemia, three with essential reactive hypoglycaemia, and one with hypoglycaemia due to hypopituitarism were also studied.

Control subjects were healthy laboratory personnel, or non-obese, non-diabetic hospital inpatients free from endocrine, hepatic, or metabolic disease. In addition five healthy, obese, non-diabetic subjects were investigated.

Glucagon tests were carried out on patients and controls maintained on a high carbohydrate diet by one of the following procedures.

1 INTRAMUSCULAR GLUCAGON TEST With the subject recumbent after an overnight fast 1 mg glucagon was given by intramuscular injection. Capillary blood for glucose determination was collected from the warmed ear lobe or finger tip before the injection and at five, 10, 15, 20, and 30 minutes and at half-hourly intervals thereafter for two to three hours. If neuroglycopenic symptoms developed the test was terminated either by

glucose or a second injection of glucagon. Preliminary results have been reported (Marrack *et al.*, 1961).

2 INTRAVENOUS GLUCAGON TEST With the subject recumbent after an overnight fast, 1 mg glucagon was injected intravenously over a period of one to two minutes. (Three milligrams of glucagon was given to two patients with insulinoma (A.P.; J.D.), one of whom (J.D.) was receiving cortisone.) Venous blood for glucose and insulin assay was collected before and at five, 10, 15, 20, 30, 45, and 60 minutes after the glucagon injection. (The important five, 10, and 15-minute samples were not always collected as some of the patients with insulinoma were studied before the dynamics of the plasma insulin response to glucagon administration (Samols, Marri, and Marks, 1966) was fully appreciated.)

Blood glucose was measured by glucose oxidase (Marks, 1959) and insulin by radio-immunoassay (Samols and Bilkus, 1964). Intravenous glucose, tolbutamide, oral l-leucine, and glucose tolerance tests were carried out as previously described (Marks and Rose, 1965) and the results interpreted according to established criteria (Samols and Marks, 1963; Samols and Marks, 1965; Samols, 1965; Floyd, Fajans, Knopf, and Conn, 1964).

INTRAMUSCULAR GLUCAGON: CAPILLARY BLOOD GLUCOSE The results of the intramuscular glucagon test in 33 control subjects and 13 patients with hyperinsulinism due to insulinoma are shown in Table I. In control subjects capillary blood glucose concentration rose from the mean fasting level of $69 \text{ SD} \pm 10.2 \text{ mg/100 ml}$ to a peak of $136 \text{ SD} \pm 16 \text{ mg/100 ml}$ after 45 minutes, and by 150 minutes had returned to slightly below the fasting value (mean $65 \text{ SD} \pm 10 \text{ mg/100 ml}$). In none did the blood

glucose concentration fall below 40 mg/100 ml and neuroglycopenic symptoms did not occur.

In nine out of 13 patients with hyperinsulinism fasting capillary blood glucose concentrations were low (*ie* less than 50 mg/100 ml) but in all except one patient (T.D.) there was a normal rise of 35 to 95 mg/100 ml after intramuscular glucagon. Concentrations were maximal after 15 to 45 minutes and fell to or below the fasting level by three hours. In each case at least one late blood glucose concentration of 40 mg/100 ml or less was recorded. During the second half of the test neuroglycopenic symptoms were common and in three of the patients were sufficiently severe to necessitate terminating the test prematurely. Coma developed after 90 minutes in W.K. on the first occasion he was examined. He was re-examined four years later with the intravenous test. Similar results were obtained, hypoglycaemic coma (blood glucose concentration of less than 20 mg/100 ml) again developing after 90 minutes. At laparotomy, no tumour was found and hypoglycaemia and hyperinsulinaemia persisted postoperatively.

In one patient (F.S.) both capillary and venous blood samples were collected. The results (Fig. 1), which show a normal rise in capillary but a subnormal rise in venous blood glucose concentration, are compatible with the response of venous blood glucose to intravenous glucagon, recorded below. Figure 1 also shows the response of capillary and venous blood glucose to an oral glucose load in this patient.

The capillary blood glucose response to glucagon

TABLE I

CAPILLARY BLOOD GLUCOSE CONCENTRATIONS (mg/100 ml) DURING INTRAMUSCULAR GLUCAGON TEST IN 13 PATIENTS WITH INSULINOMA

Subject	Sex/ Age	Capillary Blood Glucose Concentrations (mg/100 ml)											
		0 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	90 min	120 min	150 min	180 min
Benign insulinoma													
F.S.	F 48	53	79	92	100	95	89	67	63	14	26		
J.T.	M 34	42		54		98	106	96	73	42	33	29	
J.D.	F 32	22		63		86	105	102	86	48	20	14	15
P.I.	M 59	28				67		90	86	67	46	32	29
T.D.	F 42	57			50		60	35	40	50	50	20	
I.W.	M 58	23			48		61	44	28	18	16		
W.B.	M 47	31					85		51	25	15	15	
E.N.	F 59	39		76		101	118	132	115		40	33	42
A.B. ¹	F 59	78					91		93	85	70	56	48
M.C.	F 48	10	28	50	59	85	97	74	44	11	3		
Malignant insulinoma													
P.Mc.	M 42	23			70		95	95	80	53	39	28	
P.M.	M 63	29				59	66	76	80	73	62	50	27
Presumed insulinoma													
W.K.	M 58	57		83		107	112	108	73	15			
33 control subjects		69					125	136	120	92	75	65	64
(mean $\pm$ SD)		± 10.2					± 14.4	± 16.0	± 12.7	± 9.3	± 11.2	± 10.3	± 6.0

¹Blood sugar measured by non-specific method.

FIG. 1. Capillary and venous blood glucose concentrations in a patient (F.S.) with benign insulinoma during (left) intramuscular glucagon and (right) oral glucose tolerance tests. Note the much larger capillary-venous difference and more profound 'reactive' hypoglycaemia during the glucagon than during the glucose test.

FIG. 2. Venous blood glucose (above) and plasma insulin levels (below) during intravenous glucagon tests in 14 patients with hyperinsulinism due to insulinoma. (Blood glucose values shown $\bigcirc$ — $\bigcirc$ were obtained in patients who received 3 mg glucagon intravenously.)

was normal in two patients with essential reactive hypoglycaemia, and neuroglycopenic symptoms did not occur. In three patients with hepatogenous hypoglycaemia due to cirrhosis, the rise in blood glucose concentration was subnormal after intramuscular glucagon and secondary hypoglycaemia was absent. None of the patients experienced symptoms during the test.

INTRAVENOUS GLUCAGON: VENOUS BLOOD GLUCOSE Blood glucose and plasma insulin assays during the first 60 minutes of the intravenous glucagon test in 14 patients with hyperinsulinism due to insulinoma are shown in Figure 2. Also shown for comparison are the results obtained in 10 control subjects, the shaded area encompassing the upper and lower limit of observed values.

In seven out of 14 insulinoma patients the plasma insulin response to intravenous glucagon was excessive (here defined as more than twice the upper limit of normal, which is taken as $130 \mu\text{U/ml}$) and supranormal in a further three, and probably accounts for the subnormal rise in venous blood glucose concentration.

In controls mean fasting plasma insulin was $19 \mu\text{U/ml}$ ($\text{SD} \pm 5$) and rose within five minutes following intravenous glucagon (1mg) to a peak of $74 \mu\text{U/ml}$ ($\text{SD} \pm 26$) and fell gradually thereafter towards fasting values. At 10 minutes mean plasma insulin was $62 \mu\text{U/ml}$ ($\text{SD} \pm 17$); at 30 minutes $35 \mu\text{U/ml}$ ($\text{SD} \pm 13$) and at 60 minutes $21 \mu\text{U/ml}$ ($\text{SD} \pm$). Mean fasting blood glucose was 67 mg/100 ml ($\text{SD} \pm 6.5$) and rose to a peak value of 109 mg/100 ml ($\text{SD} \pm 8.5$) at 20 minutes. This confirms previous evidence (Samols *et al.*, 1965a) that the maximum rise in plasma insulin after intravenous glucagon precedes the maximum rise in blood glucose.

In five healthy, non-diabetic, obese subjects, the blood glucose response to glucagon was normal but the rise in plasma insulin was supranormal (Table II) in four of them.

FIG. 3. Venous blood glucose (below) and plasma insulin levels (above) in four patients with hypoglycaemia from diverse causes (excluding insulinoma).

The results in four patients with other forms of hypoglycaemia are shown in Figure 3. In two patients with hypoglycaemia due to non-islet cell tumours the insulin response was subnormal and the blood glucose level rose normally. In one subject with essential reactive hypoglycaemia and in another with hypopituitarism both glucose and insulin responses were normal or subnormal.

TABLE II

VENOUS BLOOD GLUCOSE AND PLASMA INSULIN LEVELS (IN PARENTHESES) IN FIVE NON-DIABETIC OBESE WOMEN BEFORE AND AFTER 1MG GLUCAGON INTRAVENOUSLY

Case No.	Age/ Sex	Percentage above Ideal Body Weight	Fasting	Minutes after 1mg Glucagon Intravenously							
				5	10	15	20	30	45	60	90
1	45/F	+70	70(3)	81(88)	100(50)	114(60)	123(47)	107(48)	87(18)	79(18)	
2	57/F	+45	45(26)	56(200+)	57(183)		56(59)	64(53)	59(47)	51(33)	45(17)
3	28/F	+37	63(50)	74(186)	83(108)	99(100)	105(114)	97(79)	81(44)	71(22)	64(30)
4	42/F	+27	71(9)	76(172)	81(83)	86(63)	97(46)	101(46)	96(40)	81(21)	69(10)
5	26/F	+18	69(31)	93(143)	102(165)	104(102)	104(96)	111(69)	87(59)	75(48)	
Mean normal subjects			67(19)	82 (74)	91(60)	100(52)	109(46)	107(36)	92(27)	78(20)	

TABLE III

SUMMARY OF INSULIN RESPONSE TO PROVOCATIVE TESTS FOR HYPOGLYCAEMIA IN 14 PATIENTS WITH HYPERINSULINISM AND TWO WITH NON-ISLET CELL TUMOURS PRODUCING HYPOGLYCAEMIA¹

Patient	Age/ Sex	Diagnosis	Fasting Plasma Insulin	Plasma Insulin Response to				
				Oral Glucose	Intravenous Glucose	Oral l-Leucine	Intravenous Tolbutamide	Intravenous Glucagon
T.H.	33/M	Benign insulinoma	N or +		N		++	++
J.B.	35/M	Benign insulinoma	N or +		++	++	++	++
R.B.	41/M	Benign insulinoma					++	+
P.D.	42/F	Benign insulinoma	N or +	+				+
P.S.	47/F	Benign insulinoma	+	N	N			N
L.E.	11/F	Benign insulinoma	+			+	++	++
A.P.	36/F	Benign insulinoma	+ or ++			+	++	++
A.P.	59/F	Benign insulinoma	N or +	N or +	N	N or +	N or +	N or +
B.H.	56/M	Benign insulinoma	+					N
J.L.	65/M	Benign insulinoma	+	+				++
J.D.	58/F	Malignant insulinoma				persistently high		
E.H.	21/F	Malignant insulinoma	+	N	N	N	N or +	++
H.T.	50/M	Malignant insulinoma	+	N	-	N or +	N	N
W.K.	50/M	? Benign insulinoma	N or +				+	++
P.K.	52/M	Carcinoma of lung	N				-	-
N.M.	60/F	Fibrosarcoma	N	-	-	-	-	-

N = normal - = subnormal + = above normal ++ = excessive (ie more than twice normal)

¹In many cases provocative tests were carried out on more than one occasion, but only where these yielded substantially different results is more than one result shown.

In two patients with hepatogenous hypoglycaemia plasma insulin rose normally in response to glucagon. In one it was accompanied by a fall in venous blood glucose concentration from 74 mg/100 ml fasting, to 48/100 ml at 90 minutes, without an intervening rise.

PLASMA INSULIN RESPONSE DURING OTHER PROVOCATIVE TESTS IN INSULINOMA PATIENTS Plasma insulin responses during various provocative tests in 14 patients with insulinoma and in two patients with non-pancreatic hypoglycaemia producing tumours are summarized in Table III. Definition of the 'normal' plasma insulin response to various stimuli to insulin secretion is difficult. Some of the problems have been discussed elsewhere (Samols, 1965; Nydick, Samols, Kuzuya, and Williams, 1964; Samols and Marks, 1965; Welborn, Rubenstein, Haslam, and Fraser, 1966). These include differences due to age, sex, race, weight, and glucose tolerance, and the genetic disposition of the subject. For the purpose of this paper, and based upon our own published data and those of others using similar immunoassay techniques, we have considered a rise in plasma insulin to more than 150 μ U/ml at any time after 100 g glucose by mouth, to more than 150 μ U/ml after 25 g glucose by rapid intravenous injection, to more than 120 μ U/ml after 1 g sodium tolbutamide intravenously, or to more than 40 μ U/ml after l-leucine by mouth as abnormally high.

In all of the insulinoma patients except H.T., there was an exaggerated rise in plasma insulin in response to one or more of the stimuli, but the

response to different stimuli and to the same stimulus on different occasions was inconstant. In the main, diagnostically useful 'excessive' insulin responses were obtained with tolbutamide (five out of nine subjects tested), glucagon (seven out of 14 subjects tested), and occasionally, l-leucine. In contrast the insulin response to intravenous glucose was usually normal (four out of six subjects tested) or subnormal (one out of six subjects) and explains our failure previously (Marks and Marrack, 1962) to find the anticipated (Cleempoel, Conard, and Bastenie, 1955) increase in peripheral glucose assimilation after intravenous glucose had been given to patients with insulinoma.

The plasma insulin response to oral glucose was too variable in patients with insulinoma to permit generalizations but in some cases appeared to be dose-dependent.

In one patient (J.D.) with a malignant insulinoma, the insulin response to provocative stimuli was difficult to judge as fasting plasma insulin levels were always very high (>200 μ U/ml).

In two patients (S.M. and C.L.) with non-pancreatic neoplasms causing hypoglycaemia, the rise in plasma insulin after oral and intravenous glucose, intravenous tolbutamide, oral l-leucine, and intravenous glucagon were consistently subnormal.

DISCUSSION

The unreliability of the oral glucose tolerance test, the non-specificity of Whipple's triad and of the prolonged fast test, together with the latter

tediousness and expense, led to a search for better tests for insulinoma. Despite considerable advances in the past decade, during which the intravenous glucose tolerance (Cleempoel *et al.*, 1955; Marks and Marrack, 1962), intravenous tolbutamide (Fajans *et al.*, 1961; Marrack *et al.*, 1961) or chlorpropamide (Linquette, Fossati, Gasnault, and Luez, 1964), oral leucine (Marrack, Marks, and Rose, 1960; Schwartz, dePeyster, and Gilchrist, 1962), and glucagon (Marks, 1960; Marrack *et al.*, 1961) tests were introduced, differential diagnosis of insulin is still occasionally difficult (Cohn, Perlmutter, Silverstein, and Numeroff, 1964; Kahil, Brown, and Dobson, 1964) even with the aid of plasma insulin assays (Nydic *et al.*, 1964; Coskey and Tranquada, 1964).

The intravenous tolbutamide test is widely used for the diagnosis of insulinoma, but experience (Cohn *et al.*, 1964; Fajans, Floyd, and Conn, 1963; Cunningham, 1964; Samols and Marks, 1967) has shown that it is neither as accurate nor as specific as originally thought, although its value is much increased when plasma insulin immunoassays are carried out simultaneously with blood glucose measurements (Samols and Marks, 1963; Floyd *et al.*, 1964). Nevertheless, confusion may arise in certain cases of hypoglycaemia due to liver disease and portocaval anastomosis and in obesity with mild diabetes. In cirrhosis with portocaval anastomosis both plasma insulin and blood glucose responses to intravenous tolbutamide and oral glucose may be abnormal and indistinguishable from those of patients with insulinomas (unpublished observations). In diabetes, prolonged fasting may cause hypoglycaemia (Beck, Koumans, Winterling, Stein, Daughaday, and Kipnis, 1964) and the plasma insulin response to tolbutamide is increased (Samols, 1965) though the blood glucose response differs from that of patients with insulinoma.

The intravenous tolbutamide test though usually innocuous is not completely without danger (Davidson, 1965; Samols and Marks, 1967), and the suggestion has been made (Johnston, Goetz, and Zimmermann, 1960) that it should be reserved 'for use in doubtful cases in which it is unlikely that a tumour is present'. Whilst we do not agree with this reservation, we consider it important that both intravenous glucose and hydrocortisone (100 mg) should be at hand for terminating hypoglycaemic coma should it develop during the test (Marks, 1967). In our experience trouble is seldom encountered unless the patient is already hypoglycaemic, though not necessarily symptomatic, when the test is begun. Since in these circumstances no useful information can be obtained from the intravenous tolbutamide test unless plasma insulin concentrations are

measured concurrently, it is our practice, when hypoglycaemia already exists, to give intravenous glucose (25 g) concurrently so that the characteristic plasma insulin response (Samols and Marks, 1963; Floyd *et al.*, 1964) may be observed without risk in patients whose hypoglycaemia is due to insulinoma.

The l-leucine test (Marrack *et al.*, 1960; Schwartz *et al.*, 1962) is more specific for insulinoma than the intravenous tolbutamide test, but less sensitive. Although false negatives are commoner, false positives (*ie* a rapid fall in blood glucose and a greater than normal rise in plasma insulin) do not occur in adults unless they are taking sulphonylurea drugs. A gradual fall in blood glucose during the course of the test should not be considered a positive response.

The intramuscular glucagon test (Marks, 1960; Marrack *et al.*, 1961) for insulinoma appears to be safe and useful. False negatives (*ie* a failure of secondary hypoglycaemia to develop) are uncommon and false positives are rare, but have occasionally been described in children with idiopathic hypoglycaemia of childhood. The test yields normal glucose results in essential reactive (so-called 'functional') hypoglycaemia, and in one patient examined the plasma insulin response to glucagon was also normal. There are insufficient data available to generalize on the plasma insulin response to intramuscular glucagon in insulinoma, but preliminary observations (unpublished) suggest that it is often excessive.

The intramuscular glucagon test has not been widely used for the recognition and differential diagnosis of spontaneous hypoglycaemia, possibly because it formerly lacked a rationale. Many authors have wrongly regarded it as a liver function test, and others as being similar to the oral glucose tolerance, to which it bears a superficial resemblance. In fact, glucagon directly stimulates the release of insulin by the β -cells (Samols *et al.*, 1965a; Turner and McIntyre, 1966) even in cases (*eg* insulinoma patient H.T., and many patients with maturity onset diabetes) where glucose alone is ineffective (Samols *et al.*, 1966). Moreover, whereas glucagon provokes an excessive rise in plasma insulin and secondary hypoglycaemia in most patients with insulinoma, intravenous glucose rarely does. Oral glucose, which stimulates glucagon secretion (Samols, Tyler, Marri, and Marks, 1965b), as well as causing hyperglycaemia, is more variable in its effect.

Although some patients with insulinoma are obese, the proportion which in our experience exceeds the ideal body weight by 25% or more is small, despite reports to the contrary in the literature. It has previously been shown that plasma insulin

levels often rise excessively after oral and intravenous glucose (Karam, Grodsky, and Forsham, 1963) or intravenous tolbutamide (Samols, 1965) in patients with simple obesity. The present results likewise indicate an excessive insulinaemic response to glucagon in obesity. This theoretically limits the value of plasma insulin determination in response to provocative stimuli in markedly obese patients, but confusion in the recognition of hyperinsulinism or in the differential diagnosis of spontaneous hypoglycaemia is unlikely to arise in practice if plasma insulin and blood glucose results are collated, because hypoglycaemia does not occur in the obese despite the high plasma insulin levels.

Examination of data published by others (Yalow and Berson, 1961; Floyd *et al.*, 1964) reveal cases of insulinoma in which a small or moderate rise in blood glucose levels following glucagon injections was associated with inordinately large rises in plasma insulin. Similarly exaggerated plasma insulin responses to glucagon have been observed by Stimmler (personal communication) in two patients with insulinomas and by Davidson (1965) in one. Normal plasma insulin responses to glucagon in two of three cases of insulinoma reported by Floyd and his associates (1964) were possibly due to errors of timing since five- and 10-minute specimens were not collected, though true 'non-responders' undoubtedly occur.

The present results indicate that while glucagon is unquestionably useful in the treatment of acute hypoglycaemic coma, whatever the cause, its value in the long-term treatment of spontaneous hypoglycaemia should be considered in each individual with respect to its insulinogenic effect.

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