revealed curves of normal contour with a slight decrease in amplitude; at this stage the B.U.N. was 19 mg./100 ml. and the creatinine clearance 105 ml./minute.

Non-functioning Kidney and Renal Agenesis (Cases 26 to 28)

The recognition of a non-functioning or absent kidney may be of importance—for example, when emergency nephrectomy is contemplated. In this situation renography is the simplest investigation for detecting the presence of functioning renal tissue. Should the renogram suggest an absent or non-functioning kidney, intravenous pyelography is indicated to exclude an ectopic kidney. Fig. 6 shows the renogram from a patient with left renal agenesis. These tracings should be compared with those illustrated in Fig. 7; the intravenous pyelogram from this patient showed a calcified tuberculous pyonephrosis of the left kidney without evidence of renal function; although the renogram showed gross depression of function on the left side, comparison with a simultaneous record obtained over the heart failed to show the progressive downward gradient of the heart curve, thus indicating persistence of some function. In Case 25, a prospective donor for renal transplantation, the finding of normal symmetrical renogram tracings in addition to normal total renal clearances and normal nephrograms and pyelograms was further evidence for the presence of two normally functioning kidneys.

Discussion

¹³¹I-hippuran renography is shown to be a rapid method of detecting obstruction of the upper urinary tract. Although delay in the disappearance of radioactivity from the renal area is invariably present in obstruction, it may also occur when there is dilatation of the collecting system or a marked diminution in urine flow; thus the renogram may show the features of obstruction in chronic pyelonephritis with caliceal clubbing and in acute circulatory failure.

In their original description of radioisotope renography using ¹³¹I-"diodrast," Taplin *et al.* (1956) illustrated tracings obtained from animals with ureteric obstruction and described similar curves in patients with renal colic. The renographic findings associated with dehydration, renal ischaemia, and acute renal failure have also been described in animals using ¹³¹I-diodrast (Taplin *et al.*, 1956; O'Connor *et al.*, 1961). Among the various ¹³¹I-labelled compounds which have been used for renography hippuran has proved most satisfactory (Nordyke *et al.*, 1960; Whitley *et al.*, 1961; Johnson *et al.*, 1961); investigators using this compound have described characteristic changes in the renograms of experimental animals and man with induced physiological changes or structural lesions (Nordyke *et al.*, 1960; Meade and Shy, 1961; Klapproth *et al.*, 1962). In this study similar renographic findings have been described in analogous clinical situations, and these are shown to be of diagnostic importance.

Summary

The results of ¹³¹I-hippuran renography are reported in 30 patients with various urological disorders.

In renal colic ureteric obstruction is shown by delayed excretion of the radioisotope-labelled compound, and the subsequent course of the obstruction can be followed by serial renograms. When persistent obstruction causes impairment of renal function this is also shown by changes in the renogram.

By revealing a renal abnormality the renogram often yields diagnostic assistance in patients with abdominal or back pain of indeterminate cause. The differentiation of anuria due to recent ureteric obstruction from that of acute renal failure can be made readily by renography; the rising curves of normal or increased amplitude characteristic of obstruction contrast with the flat curves of low amplitude found in acute renal failure. The renogram can assist in the recognition of a non-functioning or absent kidney.

It is concluded that ¹³¹I-hippuran renography is a convenient simple test of considerable value in the investigation and management of patients with a variety of urological disorders.

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INSULIN ASSAY IN INSULINOMAS

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The pre-operative diagnosis of insulin-secreting islet-cell tumours of the pancreas (insulinomas) has in the past depended on a number of empirical tests. The demonstration of Whipple's triad and the tolbutamide, glucagon, and leucine tests contribute to the diagnosis, but errors still occur.

It was hoped that a reliable insulin assay would permit the detection of insulinomas with certainty, but methods measuring insulin-like activity (I.L.A.) have given inconsistent results (Wright, 1960). Recently a specific immunoassay for insulin (Yalow and Berson, 1960) has become available. In this paper we record our observations on the use of this specific insulin assay in patients with insulinomas.

Materials and Methods

The normal range for plasma insulin was determined in 100 fasting control subjects and the normal plasma insulin response to tolbutamide in 30 control subjects.

Ten patients with surgically proved insulinomas were studied. The tumour was benign in eight and malignant with widespread metastases in two (Cases 6 and 7). Plasma insulin concentrations were measured by the radio-immunoassay technique of Yalow and Berson (1960). Blood glucose was measured by a glucose oxidase method (Marks, 1959) in eight cases, and as total reducing substance in two.

Blood samples for insulin and glucose estimations were drawn either after an overnight fast or when hypoglycaemia had developed during fasting. In some patients insulin levels were also measured after oral or intravenous glucose, after intravenous sodium tolbutamide (1 g.), after oral leucine (0.15 g./kg. body weight), and during pancreatic surgery.

Results

Fasting Plasma-Insulin Levels.—The range for fasting plasma insulin in 100 control subjects was 2–63 micro-units (μ u.) of insulin per ml., with a mean value of 19 μ u. and S.D. ± 7.5 . The results of fasting insulin assays in 10 patients with insulinomas, together with the relevant blood-glucose concentration, are shown in Table I. In eight cases more than one value, representing blood specimens drawn on different days, are shown. In 9 of the 10 patients there was at least one high fasting plasma-insulin level; but in four of these (Cases 1, 2, 4, and 9) there were occasions when hypoglycaemia was not accompanied by a very high plasma insulin (see Addendum). In one patient (Case 10) three plasma samples, each drawn during hypoglycaemia, were

TABLE I.—Fasting Plasma Insulin and Blood-glucose Levels in 10 Patients with Insulinomas

Case No.	Glucose (mg./100 ml.)	Insulin (μ u./ml.)	Case No.	Glucose (mg./100 ml.)	Insulin (μ u./ml.)
1	32	29	5	20	140
	21	41		52*	189
	23	105		52*	214
	20	37	6	68	47
	12	45		26	121
2	20	51	7	28	146
	32	63		34	89
	20	71	8	30	60
	12	119		32	65
	15	124	9	24	119
	32	134		36	31
3	30	138		13	43
	30	140	10	32	46
	27	163			
4	58*	50	Normal range	50–90	Mean 19
	53*	52		60–110*	S.D. ± 7.5
	45*	121			

* Blood sugar measured as total reducing substance.

FIG. 1.—Case 2. Effect of glucose on plasma insulin. Glucose, 50 g., was given orally at 0 minutes.

assayed and all showed a normal insulin content—this was the only subject with proved insulinoma in the present series in whom a high fasting plasma insulin was never demonstrated. In Cases 4 and 6 the true blood-glucose levels were probably lower than the blood sugar measured as total reducing substance.

Effect of Glucose.—The effect of glucose on plasma insulin was studied in Cases 2, 5, and 10. The plasma insulin and glucose levels in Case 2, before and after 50 g. of glucose orally, are shown in Fig. 1. There was a high fasting plasma insulin but no excessive rise in plasma insulin associated with the increase in blood glucose. The glucose response was normal. However, there was no rise in plasma insulin with hyperglycaemia in the other two patients (Cases 5 and 10), who received glucose at the rate of 40 g./hour by infusion for three hours (Table II).

TABLE II.—Effect of Glucose on Plasma Insulin

Case No.	Fasting		After 3 Hours I.V. Glucose	
	Glucose (mg./100 ml.)	Insulin (μ u./ml.)	Glucose (mg./100 ml.)	Insulin (μ u./ml.)
10	13	43	218	45
5	20	146	252	135

Effect of Tolbutamide.—Plasma-insulin levels were measured in five patients (Cases 1, 2, 4, 5, and 8) at intervals after receiving 1 g. of sodium tolbutamide intravenously. The results are shown in Fig. 2. The mean rise and normal range of plasma insulin in 30 control subjects treated similarly are also shown. In each of the five patients with insulinomas the rise in plasma insulin after tolbutamide was greater than was observed in any control subject. In two the blood-glucose response was typical of that described by Fajans *et al.* (1961) in patients with insulinomas. In one (Case 2) the test had to be terminated after 25 minutes when coma developed. In three patients in whom observations were more prolonged, plasma insulin decreased although the hypoglycaemia persisted. In

FIG. 2.—Effect of tolbutamide on plasma insulin. Tolbutamide, 1 g., was given intravenously at 0 minutes.

Case 2 the tolbutamide test was repeated after surgical removal of an insulinoma. On this occasion the glucose and insulin responses were normal.

Effect of L-Leucine.—Plasma insulin levels were measured in three patients (Cases 2, 4, and 10) after the oral administration of L-leucine. In Case 2 before surgery there was a prompt fall in blood glucose, resulting in convulsions within 25 minutes of the dose. The increasing hypoglycaemia was associated with a rise in plasma insulin (Fig. 3). When the test was repeated after surgical removal of an insulinoma, blood glucose and insulin levels showed a normal response following L-leucine administration. In Cases 4 and 10, despite only a moderate fall in blood glucose after L-leucine, there was a definite rise in plasma-insulin levels (Fig. 3).

FIG. 3.—Effect of L-leucine on plasma insulin. L-Leucine, 150 mg./kg. body weight, was given orally at 0 minutes.

Insulin Levels in Portal Venous Blood and Systemic Venous Blood.—In Case 10 blood was withdrawn during surgery from the portal vessels draining the pancreas and simultaneously from two peripheral veins. Glucose was not administered either before or during this procedure. The portal venous blood-glucose concentration was 44 mg./100 ml. and that of the peripheral venous sample 32 mg./100 ml. The insulin concentration exceeded 518 μ u./ml. in portal venous blood, but in the peripheral blood it was only 31 μ u./ml. In two normal fasting subjects portal venous insulin levels were 46 and 54 μ u./ml.

Discussion

Until recently, only bioassay techniques were available for measuring blood insulin. However, these methods, which depend in the main on changes in glucose metabolism of animal tissues *in vivo* or *in vitro*, measure plasma insulin-like activity (I.L.A.) and are not specific for insulin (Bornstein and Lawrence, 1951; Groen *et al.*, 1952; Vallance-Owen and Hurlock, 1954; Martin *et al.*, 1958).

Elevated plasma I.L.A. has been reported in patients with insulinomas (Bornstein, 1951; Groen *et al.*, 1952; Vallance-Owen *et al.*, 1955; Randle, 1957; Martin *et al.*, 1960). Wright (1960) examined fasting plasma samples from seven patients with proved insulinomas and detected a raised I.L.A. in three. In a series of 16 patients Groen *et al.* (1958) found a high fasting plasma I.L.A. in eight patients and normal levels in the others. These workers pointed out that insulin antagonists, such as adrenaline, interfere with the bioassay for insulin. Adrenaline, which might be expected to be increased

during hypoglycaemia, would diminish plasma I.L.A. This, they thought, might account for their failure to find elevated plasma I.L.A. invariably in patients with insulinoma and hypoglycaemia.

The specific immunoassay for insulin used in the present series is not subject to interference by antagonists; nevertheless, a high fasting plasma-insulin level was not an invariable finding. In both the present series and that described by Yalow and Berson (1960) only about half of the fasting plasma samples assayed during hypoglycaemia showed significantly raised insulin levels.

Our present series supports the observation by Willebrands and Groen (1956) that raised fasting plasma I.L.A. can be demonstrated at some times in patients with insulinomas but not at others. Wright (1960) has suggested that incorrect timing of the sample might be responsible. The validity of Wright's theory has been confirmed for the first time by our recent investigations, which establish that very significant spontaneous fluctuations in plasma-insulin levels may occur over short periods of time in these patients (see Addendum). However, Yalow and Berson (1961) found normal insulin levels on repeated sampling for several hours during prolonged fasting hypoglycaemia. Another possible explanation for these results is discussed later.

The plasma-insulin response to a glucose load in patients with insulinomas (Yalow and Berson, 1961) is variable. Our results show that hyperglycaemia may or may not be associated with a rise in plasma-insulin levels. This may in part explain the variation in the glucose-tolerance curves in this disease.

All patients with insulinomas so far reported have shown a more consistent response to tolbutamide. Five patients in the present series and three studied by Yalow and Berson (1961) showed an exaggerated but transient rise in plasma insulin following tolbutamide. These effects of glucose and tolbutamide contrast with those seen in normal subjects, when the rise in plasma insulin produced by glucose is greater and more sustained than that caused by tolbutamide (Yalow *et al.*, 1960; unpublished observations).

The mechanisms of insulin release by glucose and tolbutamide are not necessarily the same. Seltzer (1962) suggests that while glucose is truly "insulogenic"—that is, that glucose not only causes release but also stimulates production of insulin by the pancreatic islet cells—tolbutamide is "merely capable of 'solubilizing' pre-formed, stored insulin and enabling it to be rapidly discharged." It is possible that while the "insulogenic" activity of glucose is impaired when islet cells become tumorous, their ability to respond to tolbutamide by releasing insulin remains intact.

The prolonged hypoglycaemia described by Fajans *et al.* (1961) after tolbutamide in patients with insulinomas may persist for several hours after the plasma-insulin levels have returned to or fallen below their fasting level. Thus high circulating plasma-insulin levels do not necessarily explain this phenomenon.

Cochrane *et al.* (1956) reported induction of hypoglycaemia as an abnormal response to L-leucine in three children and one adult. This abnormality occurs as a rare "inborn error of metabolism." It has been shown that in affected subjects leucine causes an exaggerated release of insulin (Yalow and Berson, 1960). It is now apparent that about one-third or more of all patients

with insulinomas are sensitive to leucine. After successful surgical treatment these patients lose their sensitivity. In our Case 2 and in the case of insulinoma described by Marks and Klein (1961) there was a large rise in plasma insulin following the ingestion of L-leucine which did not occur after removal of an insulinoma. Thus it would appear that in these two patients it was only the tumour cells which responded abnormally to L-leucine. The rise in plasma insulin after L-leucine in cases 4 and 10 was associated with a fall in blood glucose but not to the low levels classically seen in L-leucine-sensitive patients. We suggest that insulinomas are probably not strictly leucine-sensitive or leucine-insensitive, but that there is usually an excessive release of insulin by the hyperactive cells of the tumour, and the consequent hypoglycaemia is a matter of degree.

The insulin levels in the portal venous plasma were more than thirteen times as high as the peripheral venous plasma estimated at the same time. Insulin secreted into the portal venous system will normally be considerably diluted in the systemic circulation. Also it has been shown that the liver itself removes a significant amount of the insulin perfusing it (Samols and Ryder, 1961). Hypoglycaemia inhibits insulin secretion by normal islet cells (Metz, 1960), yet despite hypoglycaemia the portal venous insulin, secreted by the autonomous tumour, was very high.

Although the action of insulin on the liver is controversial, there is now good evidence that portal venous insulin decreases the hepatic glucose output (Madison *et al.*, 1960). An insulinoma may thus produce hypoglycaemia predominantly by decreasing hepatic glucose output. The simultaneous removal of insulin by the liver would account for normal systemic insulin levels which may be observed during hypoglycaemia in some patients with insulinoma.

A raised fasting plasma-insulin level accompanying spontaneous hypoglycaemia is suggestive, though not necessarily diagnostic, of insulinoma. For while fasting plasma insulins are generally normal in other varieties of organic hypoglycaemia (Berson and Yalow, 1961; unpublished observations) exceptions have been observed in retroperitoneal fibrosarcomata and idiopathic hypoglycaemia (Whitney and Heller, 1961; Oleesky *et al.*, 1962; Berson and Yalow, 1961).

A normal plasma insulin does not exclude the diagnosis of an insulinoma. Our results suggest that multiple assays may assist diagnosis by increasing the chance of finding a high insulin level. Additional help may be obtained by measuring insulin levels after intravenous tolbutamide.

Summary and Conclusions

Plasma insulin was assayed by an immunoassay technique in 10 patients with insulinomas. In nine patients high fasting plasma-insulin levels were demonstrated on one or more occasions, but normal fasting plasma insulins were also found. In the tenth patient none of three fasting plasma samples assayed revealed a high plasma insulin. Normal insulin levels during hypoglycaemia may be explained in part by rapid spontaneous fluctuations of insulin levels, and in part by hepatic removal of insulin.

There was a consistent excessive rise in plasma insulin after the intravenous injection of tolbutamide and after

ingestion of L-leucine. This abnormal, exaggerated insulin response was abolished by removal of the tumour.

The plasma-insulin response to glucose was variable.

The value of plasma-insulin assays in the diagnosis and differential diagnosis of insulinomas is discussed.

ADDENDUM.—Insulin studies in an additional four patients with proved insulinomas have established that significant spontaneous fluctuations in fasting plasma insulin may occur over short periods—for example, plasma insulin fell from 163 to 29 μ u./ml. within 40 minutes. All four patients had high fasting insulin levels at some stage. Changes of more than 20 μ u. of insulin per ml. over 20-minute intervals, never seen in fasting normal subjects, were not infrequent in these patients. This observation, which has not been previously described, is not only important diagnostically, but also cautions against facile interpretations of the plasma-insulin response to various substances. Nevertheless, our additional investigations have confirmed that tolbutamide and L-leucine consistently provoke an excessive rise of plasma insulin. The insulin response to tolbutamide was particularly helpful when the blood-glucose response was invalid because of fasting hypoglycaemia. Glucose was again followed by a variable plasma-insulin response, as was glucagon.

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