Insulin Secretion by Insuloma and Normal Pancreas Slices

Studies in a Patient with Multiple Endocrine Adenomata

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SUMMARY

A case of multiple endocrine adenomata and polycystic kidneys is reported. Functioning adenomata of the parathyroids and pancreas were removed surgically and a pituitary adenoma was associated with galactorrhea and amenorrhea.

Serial plasma glucose and insulin determinations during sixteen months of observations confirmed the temporary efficacy of tumor resection and partial pancreatectomy for islet cell adenomata. The suspected presence of multiple pancreatic islet cell adenomata, not removed at surgery, was confirmed when hyperinsulinism and hypoglycemia returned several months postoperatively. With benzothiadiazine therapy, plasma glucose levels rose and insulin concentrations fell, suggesting that these drugs exert a diabetogenic effect primarily through inhibition of insulin secretion. This effect does not appear to be mediated through an adrenal mechanism since it was seen in spite of bilateral adrenalectomy in this patient.

Evidence is presented which conflicts with the usual explanation for diabetic glucose tolerance curve in patients with functioning islet cell adenomata. In vitro studies with apparently uninvolved pancreas slices revealed that insulin secretion was stimulated by increasing concentrations of glucose. Insulin release occurred in vivo following oral glucose. Portal venous plasma insulin levels fell markedly only after resection of the normal tail of the pancreas. These findings indicated that there was no suppression of insulin output from the apparently uninvolved pancreas by the hyperinsulinemia associated with the tumor. During glucose tolerance testing, hyperglycemia was associated with hyperinsulinemia, a finding which supports a peripheral insensitivity to insulin's hypoglycemic action as a probable mechanism for the diabetic glucose tolerance in this patient.

Finally, evidence is presented that apparently uninvolved pancreatic tissue responds in vitro to mannose, glucose, leucine and tolbutamide. The response to glucose can be blocked by 2-deoxyglucose, an agent which blocks the metabolism of glucose 6-phosphate.

These findings suggest that insulin release in man is related to glucose phosphorylation or some product of glucose 6-phosphate metabolism. DIABETES *17*:83-89, February, 1968.

A thirty-seven-year old Puerto Rican woman was observed for four years for four major conditions:

1. Pituitary enlargement with amenorrhea and galactorrhea.
2. Multiple functioning parathyroid adenomata.
3. Multiple functioning pancreatic beta cell adenomata.
4. Polycystic kidneys.

This paper presents observations in this patient concerning insulin secreting activity of the islet cell adenomata in vivo, and of apparently uninvolved pancreatic tissue in vitro. The authors are not aware of any reports of endocrine adenomatosis and polycystic kidneys in the same patient.

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CASE REPORT

First admission: Parathyroidectomy. The patient, a thirty-seven-year old Puerto Rican woman, was admitted for the first time in December 1962, because of amenorrhea. Her menarche was at age thirteen and she had had cycles of twenty-eight days. Her first, third and fourth pregnancies (1953, 1956 and 1959) terminated with stillborn infants. In 1954, she had her only living child, a son. Following the fourth pregnancy, she had three menstrual periods and then complete cessation of menses. In the next three years she noted lactation from both breasts, weight gain from 123 to 166 pounds, decreased libido, and frequent nocturnal headaches. Past history was otherwise negative. Her deceased

father had a history of peptic ulcers, and her mother a history of kidney stones. The son was living and well.

Physical examination revealed moon facies and truncal obesity. Blood pressure was 200/130 mm.Hg. Milk could be expressed from both breasts. The vaginal mucosa had evidence of estrogenic stimulation and a smear showed estrogen effect. The remainder of the physical examination revealed no abnormalities.

There was radiographic evidence of polycystic kidneys. The serum calcium was 13.4 mg. per 100 ml. and serum phosphate 2.9 mg. per 100 ml. Tubular reabsorption of phosphate was 60 per cent and phosphate clearance was 28 ml. per minute. Bone survey and skull films were normal. After administration of dexamethasone (0.5 mg. every six hours for eight doses), urinary 17-ketogenic steroid levels fell from 41.8 mg. to 11.8 mg. per 24 hr. Ketogenic steroid excretion rose from 41.4 mg. to 72 mg. on the day of an eight-hour infusion of 25 U. ACTH. Thyroid function and urinary gonadotropins were normal. Fasting plasma glucose level was 72 mg. per 100 ml., and glucose tolerance was slightly impaired (figure 1). The parathyroid glands were operated upon and three adenomata found. The postoperative course was uneventful except for transient hypocalcemia. She was discharged four months after admission.

Second admission: Adrenalectomy. She returned, in April 1963, for adrenalectomy. Laboratory values were unchanged except for a mild anemia. Baseline urinary 17-ketogenic steroid levels were 31.4 mg. and fell to 7.7 mg. per 24 hr. on the second day of administration of dexamethasone (0.5 mg. every six hours for eight doses). Aortography and perirenal air insufflation suggested a left suprarenal mass. A total bilateral adrenalectomy was performed in May 1963. The glands contained small cortical nodules measuring from 0.1 to 0.4 cm. in diameter. The left gland weighed 9.8 gm. and the right 2.7 gm. Histological sections showed hyperplastic cortical nodules. The postoperative course was uneventful and she was discharged on maintenance doses of cortisone and 9 alpha-flourohydrocortisone.

Third admission: Hypoglycemia. In July 1964, she was readmitted because of recurrent symptoms of headache, hunger and weakness, relieved by food ingestion. Plasma glucose concentration fell from 85 mg. per 100 ml. to 25 mg. per 100 ml. one hour after the intravenous injection of tolbutamide. At this time she had symptoms of hypoglycemia which disappeared with 100 gm. of glucose given orally. Serum growth hormone level rose from 2.0 mμg. per ml. to 8.0 mμg. per ml., one hour after tolbutamide and fell to 2.0 mμg. per ml. one hour after glucose administration.* Insulin assays were not performed on these samples. She was discharged on a diet high in protein content administered as six equal feedings daily for a suspected pancreatic islet cell adenoma.

Fourth admission: Pancreatectomy. In September 1965, she was readmitted because of recurrent episodes of dizziness, sweating, headaches, craving for sweets and emotional lability. Symptoms were relieved by food or sugar ingestion. Hospitalization was prompted by a grand mal seizure and coma. She became responsive immediately after glucose administration. Plasma glucose concentration before intravenous sugar was given was 35 mg. per 100 ml. Physical examination was unchanged. Skull films showed an increase in the size of the sella turcica with erosion of the floor of the pituitary fossa. Visual field examinations, thyroid function tests, brain scan and spinal fluid examinations were negative.

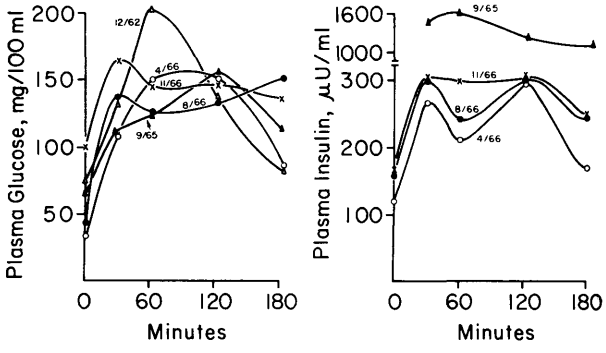

FIG. 1. Plasma concentrations of glucose and insulin after 100 gm. of oral glucose in a patient with functioning islet cell adenomata.

Many random fasting plasma glucose concentrations[2] were between 35 and 40 mg. per 100 ml. Fasting serum growth hormone levels were 6.0 mμg. per ml. at these glucose concentrations. The results of a glucose tolerance test were in the range of mild diabetes and associated with high plasma insulin[3,4] concentrations (figure 1). After 1.0 gm. of intravenous tolbutamide she had progressive sweating, tachycardia, hunger, disorientation, agitation and semistupor, necessitating discontinuance of the test forty-five minutes after the injection. The fasting plasma glucose level of 63 mg. per 100 ml. fell to 20 mg. per 100 ml. in thirty minutes and was associated with a plasma insulin rise from 155 μU. per ml. to over 600μU. per ml. Pancreatic exploration was carried out and, at the junction of the body and tail of the pancreas, a bluish cyst, 4 cm. in diameter, was found. Upon palpation of the head of the pancreas a second cystic mass was found. The tail of the gland had a diffuse nodularity. The first cystic tumor was excised; this showed islet cell adenomata in the cyst wall. The nodularity in the tail of the pancreas suggested multiple adenomata; accordingly, this was next removed. Multiple sections of this portion of the gland showed fibrosis but no evidence of adenomata. Studies of insulin secretion by slices from the tail of the pancreas are reported below. The second cystic adenoma was aspirated, opened, biopsied and sutured to the pancreatic bed. Microscopic study showed islet cell adenomata in the biopsy specimen.

With a continuous infusion of 5 per cent dextrose in water, plasma glucose levels were elevated and fatty acid[5] concentrations were low. Portal venous insulin concentrations were unchanged after resection of the first cystic adenoma but fell after resection of the tail of the pancreas (figure 2). Systemic venous insulin levels fell approximately 50 per cent after surgery (figure 3). At the time of cyst aspiration, persistent hypotension developed and responded slowly to blood

*We are grateful to Richard Barrett, M.D. for the determinations of serum growth hormone concentrations.[1]

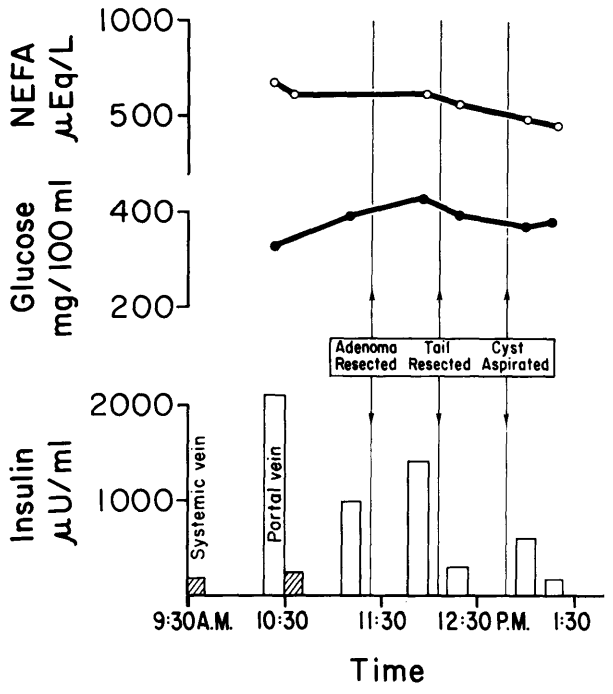

FIG. 2. Plasma concentrations of glucose, fatty acids and insulin during surgery for islet cell adenomata.

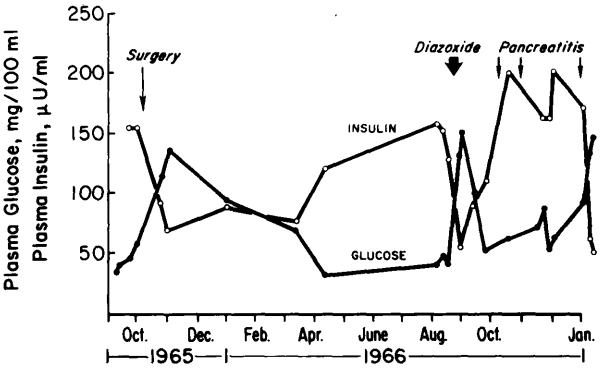

FIG. 3. Serial determinations of plasma glucose and insulin levels after an overnight fast in a patient with functioning islet cell adenomata.

and fluid administration. For this reason resection of the second adenoma was not attempted. Fluid obtained from the cystic adenoma which was not resected had an insulin concentration of 15 mU. per ml.

Three weeks after surgery the patient developed a left subdiaphragmatic abscess which was drained. Three weeks later a second subdiaphragmatic abscess was drained. Three months after admission she became asymptomatic and afebrile and was discharged to follow-up care.

Two family members, her mother and son, were screened for multiple endocrine adenomata. The mother had a fasting plasma glucose concentration of 96 mg. per 100 ml., insulin level of 21 μU. per ml., and a serum calcium and phosphate of 10.1 and 3.4 mg. per 100 ml. respectively. The son had the following values; serum glucose 90 mg. per 100 ml., insulin 19 μU. per ml., calcium 10.9 mg. per 100 ml. and phosphate 3.5 mg. per 100 ml. These screening tests suggested no familial occurrence of this syndrome at that time.

Fifth admission: Diazoxide therapy. Plasma glucose levels rose and insulin concentrations fell postoperatively, and the patient remained asymptomatic until April 1966 (figure 3). Over the next four months, however, she noted an increase in nocturnal headaches, dizziness and craving for sweets which prompted rehospitalization in August 1966. Plasma glucose and insulin levels had returned to preoperative ranges. Glucose tolerance tests performed in April and August were again mildly diabetic and associated with an initial rise in plasma insulin levels followed by a sustained elevation (figure 1). Following 600 mg. of diazoxide* given intravenously over a thirty-minute period plasma glucose rose from 33 to 112 mg. per 100 ml., and plasma insulin fell from 230 to 160 μU. per ml. She was placed on trichlormethiazide 2 mg. q6h and diazoxide 200 mg. q8h with relief of hypoglycemic symptoms and a return of plasma glucose and insulin levels to nearly normal ranges (figure 4). However, because of azotemia (figure 4) associated with nausea and vomiting, the thiazides were discontinued.

Final admissions: She was readmitted in October and November, 1966 and January 1967, because of recurrent attacks of abdominal pain associated with elevated serum and urine amylase concentrations. Abdominal scars were hyperpigmented.

*Obtained through the courtesy of Howard N. Schwartz, M.D., Schering Corporation, Bloomfield, New Jersey.

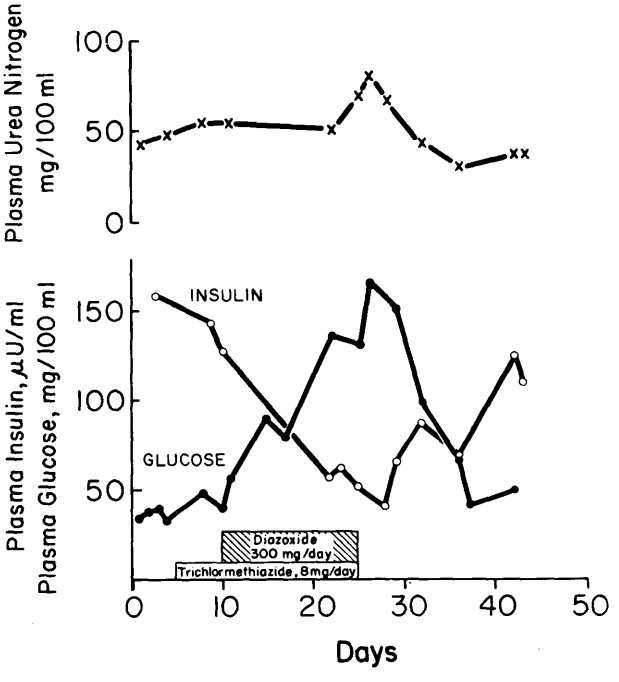

FIG. 4. Plasma levels of glucose, insulin and urea nitrogen in a patient with functioning islet cell adenomata treated with diazoxide and trichlormethiazide.

Upper gastrointestinal tract radiographs revealed a 10 cm. cystic mass of pancreatic origin which compressed the greater curvature of the stomach. Symptoms responded poorly to medical management and fasting glucose concentrations remained nearly normal off of diazoxide therapy. Glucose tolerance and insulin concentrations were unchanged (figure 1). In January 1967, following an attack of pancreatitis, fasting hyperglycemia was associated with a fall in plasma insulin concentrations (figure 3).

STUDIES ON INSULIN SECRETION IN VITRO

Methods. In vitro studies were performed on pancreas slices from the tail of the pancreas within two hours after surgical resection. Single slices of pancreas weighing from 60 to 150 mg. were incubated in flasks containing 4 ml. of Krebs-Ringer bicarbonate buffer and glucose (100 and 160 mg./100 ml.), tolbutamide (50 mg./100 ml.), leucine (100 mg./100 ml.), 2 deoxyglucose (600 mg./100 ml.), mannose (600 mg./100 ml.), or fructose (300 mg./100 ml.). Glucose (100 mg./100 ml.) was added to the flask with tolbutamide and leucine. Glucose (300 mg./100 ml.) was added to the flask containing 2 deoxyglucose. Two or three slices were incubated at each concentration in separate flasks for thirty minutes in a metabolic shaking incubator at 37° C., with 95 per cent O_2-5 per cent CO_2 gas phase and *p*H of 7.35-7.45. Insulin[3,4] and glucose[2] concentrations were determined at the end of incubation. Insulin output was expressed as μU. per mg. wet weight of pancreas per 30 min. of incubation. Pancreas slices were stained with aldehyde fuchsin[6] after incubation.

Results. The findings in the incubation studies are given in figure 5. It is apparent that the insulin secretion evoked by 100 mg. per 100 ml. of glucose alone was increased by the addition of tolbutamide and leucine. A glucose concentration of 160 mg. per 100 ml. appeared to evoke slightly more insulin secretion than did 100 gm. per 100 ml. An increase in glucose concentration to 300 mg. per 100 ml. did not increase insulin secretion further. There was a marked decrease in insulin secretion by slices exposed to this concentration of glucose when 2 deoxyglucose was added. Mannose stimulated insulin secretion to about the same degree as glucose (100 mg./100 ml.) while fructose did not. Islet stains of the incubated slices showed that beta cell granulation appeared similar in all slices. The degree of granulation was qualitatively normal in all cases. There were no adenomata found in the incubated slices. Acinar and islet tissue had no evidence of autolysis. A second series of slices incubated one-half hour after the above slices had erratic insulin release in vitro, suggesting that insulin degradation had started to occur.

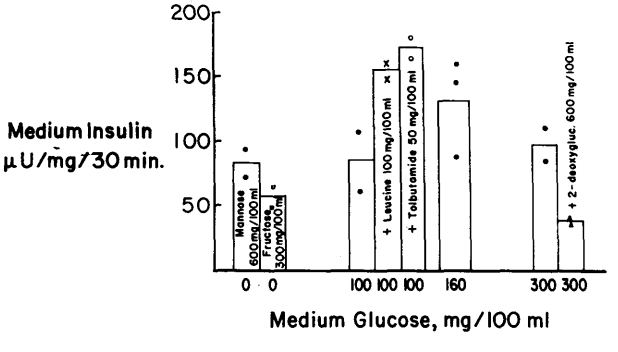

FIG. 5. Insulin output from slices of apparently uninvolved pancreatic tissue incubated in vitro as marked. For details of incubation procedures see text.

DISCUSSION

Endocrine adenomatosis. This subject has been extensively reviewed in the recent literature.[7] The findings of parathyroid, pancreatic and pituitary adenomata are typical. Hyperplasia of the adrenals is unusual. In our subject, the appearance of pituitary enlargement and pigmentation two years after bilateral adrenalectomy suggests the development of an ACTH-producing tumor, probably a chromophobe adenoma.[8,9]

Wermer[10,11] has written on the genetic aspects of endocrine adenomatosis and has suggested that the disease is transmitted by a single dominant autosomal gene with a high degree of penetrance. He postulates that there is a direct effect of an abnormal gene to stimulate cell multiplication in a variety of tissue of diverse function and origin, leading to adenomatous or excessive growth. The coexistence of endocrine adenomatosis and polycystic renal disease has not been previously reported. Dalgaard's extensive analysis of 284 patients with polycystic renal disease and their families supports the concept that polycystic kidneys are inherited as a dominant with a high degree of penetrance.[12] In view of the fact that the pituitary, parathyroids, pancreas and adrenal cortex all begin embryonic development at the sixth to eighth week, it is of interest to note that it is at about this stage of development that renal tubular differentiation takes place.[13] One is tempted to postulate that a common genetic control may have accounted for the multiple abnormalities observed in this patient. Although a high familial incidence of endocrine adenomatosis has been

reported,[11] studies of serum calcium, phosphate, glucose and insulin concentrations in two other members of this family (mother and son) were normal. The only suggestions of a familial occurrence was the history of peptic ulcer in the father and renal calculi in the mother.

Islet cell adenomata. Pancreatic islet cell adenomata were suspected on the basis of symptomatic fasting hypoglycemia and confirmed by multiple elevated fasting plasma insulin concentrations. In our laboratory, fasting plasma insulin concentrations are rarely greater than 30 μU. per ml. In normal man, portal venous insulin concentrations are 46 to 54 μU. per ml., whereas portal venous insulin levels of 164 to greater than 518 μU. per ml. have been found in patients with insuloma.[14,15] In our patient, resection of the first cystic adenoma did not alter the extremely high portal venous insulin concentration, while resection of the tail of the pancreas reduced these levels significantly. This finding implies that the normal islets in the tail of the pancreas were contributing significantly to the hyperinsulinemia observed preoperatively. In view of the fact that a second islet cell adenoma could not be resected, the failure of postoperative peripheral or portal venous plasma insulin levels to return to normal ranges was anticipated. It should be noted that the cyst fluid from this second adenoma contained insulin in a concentration of approximately 15 mU. per ml., or about 100 times the patient's plasma insulin concentration. This finding indicates that the nonresected tumor was functional.

When hypoglycemia recurred and plasma insulin levels again rose, further surgical intervention was deemed inadvisable by the surgical staff, and drug therapy was used. In accord with the results reported by others,[16-20] trichlormethiazide and diazoxide treatment produced hyperglycemia and a fall in plasma insulin levels, with a return to former concentrations when the drugs were stopped. Therapy was stopped when it increased the azotemia caused by the polycystic renal disease. These findings corroborate the studies which ascribe a major mechanism of action of diazoxide to an inhibition of pancreatic insulin release.[21,22] In view of the fact that a stimulation of adrenal medullary hormones by diazoxide has been implicated in diazoxide hyperglycemia,[23-26] it is of particular interest that our patient had been totally adrenalectomized but still demonstrated hyperglycemia and a fall in plasma insulin levels on the drug. Although extra-adrenal sites of production of catecholamines cannot be ruled out, our findings support Seltzer's contention[21] that diazoxide produces hyperglycemia by a direct effect on pancreatic insulin secretion. The adrenals do not appear necessary for this effect in man.

Pancreatic endocrine function in man. Although numerous observers have studied insulin secretion from pancreas slices in animals,[22,27] few comparable studies in man have been made. In our studies the limited number of slices dictate modest interpretation of results. Nevertheless, it appears that glucose stimulated insulin secretion and this effect was blocked by 2-deoxyglucose, an agent which is a substrate for enzymes phosphorylating glucose but is not metabolized beyond 2-deoxyglucose-6-phosphate. The metabolism of glucose 6-phosphate is blocked by 2-deoxyglucose-6-phosphate. These effects of 2-deoxyglucose agree with findings in the dog by Kilo et al.,[28] but disagree with the findings of Coore and Randle in rabbit slices[27] and Grodsky et al. in the isolated perfused pancreas of the rat.[29] Species differences in enzymes responsible for phosphorylating glucose in pancreatic beta cells may be suspected. Mannose, a sugar which is phosphorylated by glucokinase and hexokinase as is glucose, stimulated insulin secretion to a degree greater than fructose. Similar findings with mannose have been reported in the isolated perfused pancreas of the rat.[29,30] Like glucose, mannose is metabolized beyond the hexose monophosphate stage. The findings with glucose, mannose, and 2-deoxyglucose support the concept that insulin release in man is related to glucose phosphorylation or some product of glucose 6-phosphate metabolism.

Leucine and tolbutamide, two agents which may raise plasma insulin levels in patients with islet cell adenomata acted directly on the pancreas slices to induce insulin release. These results in man parallel those of Karam et al. with in vivo infusion[31] and those of Coore and Randle with rabbit pancreas slices.[27]

Following glucose loading, this patient demonstrated a mildly diabetic glucose tolerance curve in spite of an immediate increase in insulin secretion (figure 1). Plasma insulin levels were consistently above normal, although not as high as after tolbutamide, confirming the work of Floyd et al.[15] Further, only after resection of the tail of the pancreas, did portal venous plasma insulin levels fall markedly (figure 1). Slices from the apparently uninvolved pancreatic tissue demonstrated insulin secretion with glucose and other agents which have been reported to induce insulin secretion by a direct pancreatic mechanism in mammals. Assuming

that our in vitro studies accurately reflect in vivo activity, these findings suggest that the pancreatic islets in this patient responded normally to glucose and other appropriate stimuli and speaks against an hypothesis which suggests that the diabetic glucose tolerance tests were due to suppression of normal beta cell function by hyperinsulinemia.[32] The fact that direct infusion of insulin into the pancreas does not appear to inhibit insulin secretion in the normal animal[33] also mitigates against this hypothesis. It is more likely that the diabetic glucose tolerance curve in this patient was related to a persisting action of counterregulatory hormones (i.e., catecholamines) or peripheral tissue resistance in the presence of prolonged hyperinsulinemia. The former mechanism has been proposed for the temporary hyperglycemia seen after resection of an islet cell adenoma by Johnston et al.[34] Finally, the in vitro studies of apparently uninvolved pancreatic tissue with leucine and tolbutamide suggests that the plasma insulin response seen in patients with islet cell adenomata after administration of these agents may not only represent insulin stored in the neoplasm, but also in the normal pancreas. Of particular interest in this regard are in vitro studies by Field[35] in which he was unable to show that leucine or tolbutamide could stimulate release of insulin by tumor slices from islet cell adenomata which functioned in vivo.

ACKNOWLEDGMENT

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REFERENCES

[1] Yen, S. S. C., Pearson, O. H., and Stratman, S.: Growth hormone levels in maternal and cord blood. J. Clin. Endocr. *25*:655, 1965.

[2] Saifer, A., and Gerstenfeld, S.: The photometric microdetermination of blood glucose with glucose oxidase. J. Lab. Clin. Med. *51*:448, 1958.

[3] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes *12*:115, 1963.

[4] Morgan, C. R., Sorenson, R. L., and Lazarow, A.: Studies of an inhibitor of the two antibody immunoassay system. Diabetes *13*:1, 1964.

[5] Goss, J. E., and Lein, A.: Microtitration of free fatty acids in plasma. Clin. Chem. *13*:36, 1967.

[6] Gomori, G.: Observations with differential stains on human islets of Langerhans. Amer. J. Path. *17*:395, 1941.

[7] Ballard, H. S., Frame, B., and Hartsock, R. J.: Familial multiple endocrine adenoma-peptic ulcer complex. Medicine *43*:481, 1964.

[8] Nelson, D. H., and Meakin, J. W.: A new clinical entity in patients adrenalectomized for Cushing's syndrome. J. Clin. Invest. *38*: 1028, 1959.

[9] Nelson, D. H., Meakin, J. W., and Thorn, G. W.: ACTH-producing pituitary tumors following adrenalectomy for Cushing's syndrome. Ann. Intern. Med. *52*:560, 1960.

[10] Wermer, P.: Genetic aspects of adenomatosis of endocrine glands. Amer. J. Med. *16*:363, 1954.

[11] Wermer, P.: Endocrine adenomatosis and peptic ulcer in a large kindred. Amer. J. Med. *35*:205, 1963.

[12] Dalgaard, O. Z.: Bilateral polycystic disease of the kidneys. A follow-up of two hundred and eighty four patients and their families. Acta Med. Scand., Suppl. *1*, 1957.

[13] Arey, L.: Developmental Anatomy. Philadelphia and London, W. B. Saunders Co., 1940.

[14] Samols, E., and Marks, V.: Insulin assay in insulinomas. Brit. Med. J. *1*:507, 1963.

[15] Floyd, J. C., Fajans, S. S., Knopf, R. F., and Conn, J. W.: Plasma insulin in organic hyperinsulinism: comparative effects of tolbutamide, leucine and glucose. J. Clin. Endocr. *24*:747, 1964.

[16] Bleicher, S. J., Chowdhury, F., and Goldner, M. G.: Thiazide therapy in hypoglycemia of metastatic insulinoma. Clin. Res. *12*:456, 1964.

[17] Marks, V., Rose, F. C., and Samols, E.: Hyperinsulinism due to metastasizing insulinoma. Treatment with diazoxide. Proc. Roy. Soc. Med. *58*:577, 1965.

[18] Ernesti, M., Mitchell, M., Raben, S., and Gilboa, Y.: Control of hypoglycemia with diazoxide and human growth hormone. Lancet *1*:628, 1965.

[19] Lundvall, O., and Johnson, S.: Insuloma treated with diazoxide. Nord. Med. *73*:53, 1965.

[20] Fajans, S. S., Floyd, J. C., Jr., Knopf, R. F., Ruli, J., Guntsche, E. M. and Conn, J. W.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. J. Clin. Invest. *45*:481, 1966.

[21] Seltzer, H. S., and Crout, J. R.: Obliteration of insulin secretion by diazoxide and its reversal by tolbutamide. Diabetes *15*:523, 1966.

[22] Howell, S. L., and Taylor, K. W.: Effects of diazoxide on insulin secretion in vitro. Lancet *1*:128, 1966.

[23] Graber, A. L., Porte, D., Jr., and Williams, R. H.: Clinical use of diazoxide and mechanism for its hyperglycemic effects. Diabetes *15*:143, 1966.

[24] Tabachnick, I. I. A., Gulbenkian, A., and Seidman, F.: The effect of benzothiadiazine, diazoxide on carbohydrate metabolism. Diabetes *13*:408, 1964.

[25] Kvam, D. C., and Stanton, H. C.: Studies on diazoxide hyperglycemia. Diabetes *13*:639, 1964.

[26] Staquet, M., Yabo, R., Viktora, J., and Wolff, F.: An adrenergic mechanism for hyperglycemia induced by diazoxide. Metabolism *14*:1000, 1964.

[27] Coore, H. G., and Randle, P. J.: Regulation of insulin

secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. 93:66, 1964.

[28] Kilo, C., Long, C. L., Bailey, R. M., Koch, M. B., and Recant, L.: Studies to determine whether glucose must be metabolized to induce insulin release. J. Clin. Invest. 41:372, 1962.

[29] Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B., and Smith, D. F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Amer. J. Physiol. 205:638, 1963.

[30] Sussman, K. E., Vaughan, G. D., and Timmer, R. F.: An in vitro method for studying insulin secretion in the perfused isolated rat pancreas. Metabolism 15:466, 1966.

[31] Karam, J. H., Grasso, S. G., Wegienka, L .C., Grodsky, G. M., and Forsham, P. H.: Effect of selected hexoses, of epinephrine and of glucagon on insulin secretion in man. Diabetes 15:571, 1966.

[32] Lukens, F. D. W., and Ravdin, I. S.: Adenoma of islet cells of pancreas with operation and recovery. Amer. J. Med. Sci., 194:92, 1937.

[33] Colwell, J. A., and Colwell, A. R., Jr.: Effect of insulin infusion of pancreas on blood glucose. Diabetes 15:123, 1966.

[34] Johnston, R., Goetz, F. C., and Zimmerman, B.: Insulin-secreting tumors of the pancreas. Report of a case with an untoward response to tolbutamide and with a study of the possible mechanism of post-operative hyperglycemia. New Eng. J. Med. 263:1345, 1960.

[35] Field, J. B.: Clinical and biochemical studies in patients with functioning islet cell tumors. Amer. J. Med. 36:867, 1964.

Diet and the Fatty Acids in Cholesterol Esters of Plasma Lipoproteins

Differences in the fatty acid composition of cholesterol esters in various plasma lipoprotein fractions were observed by P. S. Roheim et al. (J. Clin. Invest. 42: 1277, 1963). The fatty acids of cholesterol esters in the low density lipoprotein fraction were similar in pattern to those of liver cholesterol esters. However, in the high density lipoprotein fraction, the pattern of fatty acids in cholesterol esters was very different from that of liver. The cholesterol ester in this lipoprotein fraction contained a high level of arachidonic acid and a low level of oleic acid.

L. I. Gidez, Roheim, and H. A. Eder (J. Lipid Res. 6:377, 1965) reasoned that changes in fatty acids in liver cholesterol esters caused by diet should be reflected in the fatty acids of the cholesterol esters of the low density plasma lipoproteins, if these were derived from liver cholesterol esters. Consequently, they undertook a study of the effect of diet on the fatty acid composition of cholesterol esters in liver and plasma lipoproteins in rats. Groups of male rats were fed the following diets for eleven weeks. Group 1 received a diet of rat pellets; group 2 received the rat pellet diet to which olive oil and cholesterol (1 per cent) had been added. Group 3 was fed a fat deficient diet, with added cholesterol (0.4 per cent) to accelerate the symptoms of fat deficiency.

The rat pellet diet (group 1) contained a level of 3.65 per cent fat, with the following proportions, 21 per cent palmitic, 4 per cent stearic, 18 per cent oleic, and 43 per cent linoleic. The pellet diet with added olive oil contained 14 per cent fat, with the following proportions: 15 per cent palmitic, 2 per cent stearic, 68 per cent oleic, and 12 per cent linoleic. The fat free diet contained less than 0.5 per cent fat, which was principally palmitic and oleic acids.

The rats were fed ad libitum and then sacrificed by exsanguination. Pooled samples of blood were taken from the rats in each group. Plasma lipoproteins were fractionated into very low density ($d < 1.019$), low density ($d = 1.019$ to 1.063) and high density ($d > 1.063$). Liver and plasma lipoprotein lipids were fractionated on silicic acid columns to separate the cholesterol ester fractions. The methyl esters of the fatty acids esterified with cholesterol were analyzed by gas-liquid chromatography.

Cholesterol esters in the high density lipoprotein fraction of plasma in group 1 contained predominantly linoleic acid (35 per cent) and arachidonic acid (46 per cent), with only 5 per cent oleic acid. The low density fraction contained 15 per cent oleic, 34 per cent linoleic, and 34 per cent arachidonic acid. The very low density fraction contained 16 per cent palmitic, 38 per cent oleic, 25 per cent linoleic, and 10 per cent arachidonic acid. Liver cholesterol esters contained 18 per cent palmitic, 39 per cent oleic, 25 per cent linoleic, and 7 per cent arachidonic. Thus, the composition of liver cholesterol esters was very similar to that of plasma cholesterol esters found in the very low density lipoproteins.

In group 2 (rat pellets and olive oil), the liver cholesterol esters had the following composition: 75 per cent oleic acid and 20 per cent linoleic acid, and less than 1 per cent arachidonic and palmitic acids. Choles-

(*Continued on page 95*)