EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

ASSOCIATION EUROPÉENNE POUR L'ÉTUDE DU DIABÈTE

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German Diabetes Association

At the 6th Congress of the German Diabetes Association, which took place in Düsseldorf from 14—16 May 1971 under the Chairmanship of Prof. Dr. Karl Jahnke, the following awards were presented:

1. "Paul Langerhans-Medal 1971"
   to Dr. Knud LUNDBAEK, Professor of Internal Medicine, University of Aarhus, Denmark, for his research work on diabetic angiopathy. His lecture "Growth hormone and diabetic angiopathy" was held in memorandum of Paul Langerhans.
2. "Ferdinand Bertram Award 1971"
   to Dr. Berend WILLMS, Privat-Dozent at the University of Göttingen for research on the "Regulation of gluconeogenesis".
3. "Award of the German Diabetes Association 1971"
   to Dr. Michael BERGER for his thesis "Studies on the lipolysis in human adipose tissue in vitro", carried out at the Diabetes Research Institute of the University of Düsseldorf.

Scandinavian Society for the Study of Diabetes

Abstracts

Seventh Meeting

Sandefjord, April 15–17, 1971

Studies on the antilipolytic effect of insulin in human adipose tissue

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The inhibitory effect of glucose on the mobilization of lipids from the fat depots *in vivo* has been attributed to either increased release of insulin, and/or changes in the sympathetic tone to adipose tissue. In favour of the latter hypothesis are the observations that glucose infusion suppresses the plasma-glycerol concentration as markedly in subjects with low insulin response to glucose as in subjects with normal insulin response. Previous work in this laboratory has demonstrated that the catecholamines: 1. stimulate lipolysis in isolated human adipose tissue via the beta-adrenergic receptors, and 2. inhibit this process via the alpha-receptors. The question arises

whether the antilipolytic effect of insulin in human adipose tissue is localized at the site(s) of the adrenergic receptors.
Sections of subcutaneous adipose tissue removed during surgical operation were incubated with no glucose added to the medium. Glycerol release was taken as an index of lipolysis. Physiological concentrations of insulin (10—100 μU/ml) were shown to: 1. inhibit lipolysis induced by noradrenaline, 2. if anything, stimulate lipolysis induced by isopropylnoradrenaline, 3. have no influence upon lipolysis stimulated by dibutyryl-cAMP, and 4. inhibit basal lipolysis.
The inhibitory effect of insulin on noradrenaline-induced lipolysis was reduced by increasing concentrations of the alpha-adrenergic blocking agent phentolamine in the medium.
The possible sites of the antilipolytic effect of insulin in human adipose tissue will be discussed.

The effect of insulin and some insulin derivatives on the glucose metabolism and the lipolysis in rat isolated fat cells.

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The effect of insulin, proinsulin and photo-oxidized insulin on the formation of CO_2 and lipid from ^{14}C-1-labelled glucose is compared with the antilipolytic effect against ACTH-induced lipolysis in absence of glucose. In proportion to the effect of pork insulin, pork proinsulin showed an activity of about 1 per cent with respect to both glucose metabolism and antilipolysis. Photo-oxidized insulin derivatives in which 63 per cent and 6 per cent of the histidyl residues were intact showed activities of about 35 per cent and 3 per cent respectively in both effects. Their ability to compete with ^{125}I-insulin for insulin antibodies was similarly reduced. These results are in agreement with the hypothesis that the effect of insulin on glucose transport and the antilipolytic effect in absence of glucose require the same molecular structure and are effectuated via the same receptors.

Adipose tissue fat cell size and number in relation to metabolism.

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Fat cell size was measured in different regions of subcutanoues adipose tissue. An estimate of total number of fat cells was calculated by dividing body fat, obtained by isotopic dilution methods, by a representative average fat cell weight.
In the normal region body fat correlated significantly with both fat cell number and size. With body fat > 20 kg correlations were again found with cell number although fat cell size had here levelled off on a constant, enlarged size. Taken together these results indicate that with moderately enlarged body fat (20—40 kg) fat cell size is the dominating factor contributing to increase of adipose tissue whereas with severe obesity cell number is the more important factor.
Physically training middle-aged men was followed with fat cell size determinations while decreasing in weight. When weight constant after 6—12 months they had small fat cells with a narrow range (0.3—0.5 μg). This was characteristic also of middle-aged and young, weight-stable athletes. Patients with endogenous hypertriglyceridaemia and adult-onset diabetes mellitus were, however, characterized by enlarged fat cells.
Associations were found between fasting plasma insulin or sum of insulin during glucose tolerance test and fat cell size. These associations were stronger than those between insulin and body fat. Furthermore, insulin was usually positively correlated with plasma triglycerides. No positive correlations were found with fat cell number and metabolic variables. Cholesterol and glucose tolerance did not correlate with adipose tissue factors.
It is suggested that the adipose tissue factor which is associated with metabolic disturbances is the enlargement of fat cells.

Protein kinase as a regulatory enzyme for insulin stimulation of glycogen synthesis in muscle.

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Insulin favours glycogen synthesis in muscle by promoting D→I conversion of glycogen synthetase. This enzyme is subjected to reversible phosphorylation (D-form) and dephosphorylation (I-form) reactions by the action of a kinase and a phosphatase. Since glycogen synthetase kinase has recently been identified as a cyclic AMP-dependent protein kinase, the effect of insulin on this enzyme has been investigated. Intact rat diaphragm preparations were incubated in the absence and the presence of insulin (0.1 U/ml) for 10 min in the absence of glucose. Extracts of the diaphragms were then prepared and partial purification of protein kinase was performed. Protein kinase has been shown to be converted from an inactive to an active form under the influence of cyclic AMP. Therefore, the amount of inactive form of the enzyme from the diaphragm was determined by its capacity of binding ^{3}H-cAMP as well as by the cyclic AMP-dependent phosphorylation of histone. The experiments have shown that insulin promotes an increase of the inactive form of protein kinase in the diaphragm to an extent of 50 per cent conversion. This inactivation of protein kinase by insulin promotes activation of glycogen synthetase and decreased activity of phosphorylase *b* kinase resulting in stimulation of glycogen synthesis. The effect of insulin on protein kinase seems to provide a more general mechanism for metabolic effects of insulin, such as decreased glycogenolysis, decreased gluconeogenesis, decreased hepatic production of glucose and the presence of the antilipolytic effect.

The activation by insulin of glycogen synthetase in the perfused rat liver

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Bishop and Larner have shown that insulin *in vivo* augments the independent form (I-form) of glycogen synthetase without affecting the total (I + D) activity. This net D→I conversion might in theory result either from a stimulation of the D→I phosphatase, an inhibition of the I→D kinase or a combination of these effects.
Using the perfused rat liver we have found that insulin effects a net D→I conversion, but only if the liver is first stimulated with epinephrine. The total (I + D) activity was constant during the experiment. This epinephrine-insulin antagonism was found in the 48 h starved animal as well as in the animal fed *ad libitum*. The alpha-blocking agent phentolamine (Regitin) was added to the perfusion medium in order to abolish the marked vasoconstrictor effect of epinephrine. The drug did not modify the hormone effect on the synthetase. These experiments suggest an insulin effect upon the synthetase kinase system.

The effect of anti-insulin serum and low glucose on glucokinase in rat liver studied by liver perfusion technique.

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It is now well established that the adaptation of high-K_m hexokinase in rat liver (glucokinase) is dependent on the presence of insulin as well as glucose. However, it is not yet clear which is the real "inducer" of the enzyme. Sols

et al. have shown by a liver-slice incubation technique that high glucose stabilizes the enzyme. Ruderman *et al.* have shown that anti-insulin serum given intravenously to fed rats causes a fall in the enzyme activity within 3 h. A simultaneous elevation in FFA level in plasma was found. By a perfusion technique Ruderman *et al.* also demonstrated that insulin preserved the activity of glucokinase in isolated liver.

To establish a possible correlation between the glucokinase activity and the FFA level in plasma and intracellular, 3 day fasted rats were refed a carbohydrate-rich diet. A decrease in FFA level in plasma and intracellular was observed within few hours of the refeeding period. The intracellular variation correlated inversely with the enzyme activity. However, perfusion of rat liver with anti-insulin for five hours did not affect the enzyme activity although the FFA level in perfusion medium as well as intracellular was increased significantly.

Perfusion experiments with shifting of the perfusion medium each 60 min showed that liver perfusions can be carried out for several hours with subnormal glucose concentrations without alteration in the enzyme activity, and the question of the significance of variation of glucose concentration within physiological limits can be raised.

Adrenergic regulation of basal insulin secretion.

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Vascular effects of catecholamines have been classified into α-adrenergic and β-adrenergic according to the concept of the existence of different receptors mediating vasoconstriction (alpha) and vasodilatation (beta). Pancreatic islet β-cells are also known to contain both α-adrenergic and β-adrenergic receptors inhibiting (alpha) or promoting (beta) insulin secretion. The possible physiological role of β-adrenergic stimulation by the adrenergic system is, however, unknown since the physiologically synthesized catecholamines adrenaline and noradrenaline both have been reported solely to inhibit insulin secretion. The present investigation was undertaken to test the possibility that physiological catecholamines might have a dual action on insulin secretion, i.e. also an insulinogenic effect analogous to the depressor effect of adrenaline on the vascular system, "adrenaline reversal", which can be elicited with very small concentrations of the catecholamine exciting only the β-receptors. — Administration of the specific β-adrenergic blocking agent *L*-propranolol to normal non-fasted mice decreased basal plasma insulin levels to about 50% of control values and elicited a mild hyperglycaemia. On the other hand, it potentiated the hypoglycaemic action of exogenously administered insulin in both normal and alloxan-diabetic animals. Another β-receptor blocking drug *L*-alprenolol, possessing intrinsic sympathomimetic activity, had no effect on basal plasma insulin level. Similarly, the administration of the D-isomers of the two drugs did not influence plasma insulin levels. *In vitro* incubation of liver, muscle and adipose tissue from *L*-propranolol treated animals with labelled glucose showed an increased $^{14}CO_2$ production and ^{14}C-incorporation into glycogen in muscle tissue compared to saline injected controls. No significant change was recorded with liver or adipose tissue. The α-blocking agent phentolamine increased basal plasma insulin levels and brought about a marked hypoglycaemia. After pretreatment with phentolamine, the intravenous injection of adrenaline or noradrenaline elicited a rapid and marked increase in plasma insulin concentration. *L*-propranolol abolished phentolamine-induced insulin secretion. Adrenalectomy was found to decrease basal plasma insulin levels. Moreover, the increased insulin secretion following phentolamine treatment was abolished in adrenalectomized and glucocorticosteroid-substituted adrenalectomized animals. — The results obtained indicated that adrenaline might have a dual action on insulin-secreting mechanisms analogous to its effect on the vascular system. In addition, propranolol potentiation of insulin-induced hypoglycaemia was interpreted as a result of increased glucose utilization and possibly glycogen preservation in muscle tissue.

Correlation between hyperinsulinaemia and hyperphagia in rats after ventromedial hypothalamic lesions.

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Bilateral destruction of the ventromedial hypothalamic area in adult rats produces hyperphagia and extreme obesity. Considerably elevated plasma immunoreactive insulin (IRI) levels have been reported in such animals. This hyperinsulinaemia has been explained either as a neuro-endocrine disturbance directly related to the hypothalamic operation or merely as a consequence of the altered food intake and body composition. In our experiments, plasma levels of IRI and glucose after 8 h fasting were determined in unrestrained rats equipped with chronically implanted venous catheters. Lesions were then made bilaterally in the ventromedial hypothalamic area by means of electrolysis. Food intake was restricted in order to prevent hyperphagia. An elevated IRI level with only small changes in blood glucose was demonstrated 2 days after lesions. After 5 days on restricted food intake, the animals were given free access to food. A positive correlation was observed between the body weight gain of each animal following 3 days of *ad lib.* feeding and the individual increase in the fasting IRI concentration after lesion. However, the cause of the early elevation of plasma IRI levels after ventromedial hypothalamic lesions remains to be established.

Role of glucose in arginine-induced insulin release in man.

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In five healthy subjects the plasma insulin response to intravenous arginine was markedly diminished when the amino acid was administered during insulin-induced hypoglycaemia. This inhibition of insulin release was not due to the catecholamine secretion during hypoglycaemia since, in separate experiments, infusion of a large dose of epinephrine was unable to suppress significantly the insulin response to arginine. It is concluded that the insulinogenic effect of arginine, for its expression, requires a normal blood glucose concentration.

The close interrelationship between arginine and glucose regarding insulin secretion was further illustrated by the finding that the insulin response to intravenous glucose infusion was markedly enhanced by prior administration of arginine. In experiments where hyperglycaemia was achieved through epinephrine infusion, no synergism could be observed between arginine and hyperglycaemia on insulin secretion. Immediately on cessation of epinephrine infusion a prompt and marked insulin peak was obtained. These findings indicate that synergism between arginine and glucose appears only when glucose itself is able to elicit insulin release.

We suggest that arginine stimulates insulin release by modulating the insulinogenic signal evoked by glucose in the β-cell, and not by a primary action of arginine itself on the insulin releasing machinery.

Plasma insulin (IRI) response to small doses of intravenous glucose and to tolbutamide in obesity.

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Islet cell hyperplasia and an exaggerated response of plasma immunoreactive insulin to various stimuli is

characteristic of human obesity. The purpose of the present investigation was to study the sensitivity of insulin release mechanism in obese subjects by means of small doses of intravenous glucose and by comparing the insulogenic effect of intravenous tolbutamide and of high dose of intravenous glucose.

Three successive doses of glucose (1, 2.5 and 5 g) were administered to 14 obese and 10 control subjects. In both groups the smallest dose of glucose was already sufficient to increase the level of plasma IRI within 2 min, although the content of blood glucose was elevated only minimally (average 5 mg/100 ml). The insulin responses increased progressively by increasing the dose of glucose and were greatest in the obese group.

The early responses (from 2 to 10 min) of plasma IRI to 1 g of tolbutamide and to 25 g of intravenous glucose were compared in 16 obese and 11 control subjects. Calculation of the ratio of plasma IRI response to glucose and to tolbutamide (IRI_{gluc}/IRI_{tolb}) revealed that obese subjects were more sensitive to tolbutamide than to glucose compared with control subjects.

It is concluded that the secretion of insulin is very sensitive to small changes of blood glucose levels, and that the obese subjects are more sensitive than the lean subjects. One gram of tolbutamide causes a greater insulin response than 25 g of glucose in obese but not in lean subjects.

Insulin secretion during continuous infusion of isoprenaline in normal and diabetic subjects.

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Serum insulin (IRI) and blood glucose (BG) was measured in 10 normal subjects and 7 maturity-onset diabetics given a continuous infusion of isoprenaline 2 μg/min before and during i.v. GTT with 25 g of glucose. As control, i.v. GTT was carried out during infusion of 0.9% NaCl the day before.

In the normal subjects, isoprenaline alone significantly increased IRI above the basal level ($p < 0.0025$) without a concommitant rise in BG. During i.v. GTT, serum insulin values also increased significantly ($p < 0.01$) although the BG did not differ significantly from the control values.

In the maturity-onset diabetics, isoprenaline likewise increased IRI without changes in BG, as well before as during i.v. GTT ($p < 0.05$). The increase in IRI after isoprenaline was not significantly lower in diabetics than in non-diabetics.

The effect of work load severity upon blood glucose and plasma insulin concentrations in exercising man.

E.D.R. Pruett. Institute of Work Physiology, Oslo, Norway.

During a series of metabolic studies involving healthy young male subjects in the post-absorptive state, changes in blood glucose and plasma immunoreactive insulin (IRI) concentrations were followed as the subjects exercised to, or nearly to, exhaustion at various exactly measured work loads of from 20% to 90% of the subject's previously measured maximal oxygen uptake (max $\dot{V}O_2$). Exercise at all work loads resulted in a decrease in plasma IRI concentration irrespective of the concomittant response of blood glucose concentration to the exercise. Exercise to exhaustion at 50% and 70% of the subject's max $\dot{V}O_2$ was accompanied by a decrease in blood glucose concentration to approximately 60% of the pre-exercise value. Exercise at work loads requiring utilization of 85—90% of the subject's max $\dot{V}O_2$, however, was accompanied by an increase instead of a decrease in blood glucose concentration by the time exhaustion was reached. It was concluded that during prolonged, severe muscular exercise, other endogenous mechanisms in addition to blood glucose concentration, can regulate circulating plasma IRI levels.

The rate of disappearance of infused glucose after exercise.

E.D.R. Pruett. Institute of Work Physiology, Oslo Norway.

Glucose was infused 15 min after the termination of exercise during a series of metabolic studies involving healthy young men in the post-absorptive state, exercising on the bicycle ergometer or the motor driven treadmill at exactly measured work loads of from 20% to 90% of the subject's previously measured maximal oxygen uptake (max $\dot{V}O_2$) level. The disappearance of the infused glucose and of the consequently elevated concentrations of plasma immunoreactive insulin (IRI) was different after different work loads; i.e. exercise of greater severity resulted in an increased rate of disappearance for both the glucose and the insulin. The variation in disappearance rate was not dependent upon either the total energy utilization or the total carbohydrate utilization during the exercise. In addition, the variations in glucose tolerance were found to be almost independent of the duration of the preceding exercise, in that severe exercise for as little as one minute resulted in nearly the same glucose and insulin responses to glucose infusion as did exercise to exhaustion at the same work load. On the other hand, the rate of disappearance of the infused glucose was found to depend upon the *rate* of energy utilization, or upon the *rate* of carbohydrate utilization; i.e. the severity of the exercise work load in relation to the individual's max $\dot{V}O_2$. Apparently, mechanisms which are responsible for the removal of infused glucose after exercise are activated very early in the exercise, and the magnitude of the activation appears to depend upon the relative severity of the exercise.

A growth hormone factor in human urine.

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By using a double antibody radioimmunoassay on human urine, ultrafiltrated and lyophilized, a factor is revealed which displaces ^{125}I-HGH from HGH antibody. The factor has been further investigated and it is concluded that it is not due to salts. The factor is diluted as expected in a HGH radioimmunoassay up to 1 : 16, then the concentration of the factor is below the sensitivity of the assay; The recovery of added HGH was 78, 90 and 96% respectively. By using differsnt volumes of urine for ultrafiltration and lyophilizing, the recovery was found to be ± 10% of the expected ± 10%.

By measuring plasma HGH and the factor in urine simultaneously after HGH injection in normal and hypopituitary subjects, a proportionality between plasma HGH and the factor in urine was established. The excretion of the factor was increased in acromegalia and was diminished in adult hypopituitarism. In 9 normal subjects it was found to be between 28—53 mg/24 h urine when the factor was measured in a radioimmunoassay for HGH.

The method has been used to study the plasma HGH compared with the excretion of the factor in urine in newly diagnosed diabetics before and after initial treatment. These results will be discussed.

Some aspects on the production of amyloid in the islets of Langerhans.

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Hyalinization of the islets of Langerhans is accepted by many authors as a form of amyloidosis. It is also accepted

that amyloid is produced by cells at the site of deposition. Experimental results have shown reticuloendothelial cells to be those that most probably produce amyloid. In the islets of Langerhans the amyloid is possibly produced by connective tissue cells. We have had an impression of an increase of mast cells in islets with amyloid deposits and have therefore studied this quantitatively.

In sections of pancreas from 9 patients with maturity-onset diabetes and 12 control cases the degree of amyloidosis in the pancreatic islets was measured and the mast cells were counted. In 8 diabetic and 7 control cases a varying degree of amyloidosis was shown. In the control cases without amyloidosis of the islets the intra-insular mast cell count was $19.1 \pm 3.0/mm^2$. The mast cell count in 6 control cases with slight islet amyloidosis ($< 2\%$) was $31.6 \pm 3.3/mm^2$ and this difference was significant ($P < 0.02$). In the total material an increasing number of mast cells was found as the degree of amyloidosis increased ($r = 0.75$; $P < 0.01$). The most severe amyloidosis and the largest number of mast cells were found in some diabetic cases.

It is conceivable that the mast cells are of some importance in the formation of amyloid in the pancreatic islets, but other explanations for the numerical increase in mast cells are possible.

Gastrointestinal hormones and the secretion of glucagon and insulin from the isolated, perfused canine pancreas.

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Using the isolated, perfused canine pancreas preparation, the interrelationship of the secretion of pancreatic glucagon and insulin was studied following stimulation with glucose, gastrointestinal hormones and the amino acid arginine.

The results confirm the concept of a role of pancreatic glucagon as a "hormone of glucose need" and suggest that it may be important in a moment to moment control of glucoregulation. The secretion of pancreatic glucagon was stimulated following infusion of gastrin, pancreozymin and arginine, whereas no increase was associated with secretin infusion. The magnitude of the increase was closely related to the glucose concentration present in the perfusion medium, being higher and more pronounced during perfusion with low concentrations of glucose (25 mg/100 ml or 50 mg/100 ml).

Stimulation of insulin secretion was seen following glucose, gastrin, pancreozymin, secretin and arginine. The magnitude of the increase was again closely related to the glucose concentration present, this time being higher and more pronounced during perfusion with high glucose concentrations (150 mg/100 ml).

Secretion of both pancreatic hormones always followed a biphasic response pattern after the stimuli mentioned, similar to the characteristic release pattern previously described for insulin after an increment in glucose concentration.

In order to elucidate whether endogenous pancreatic glucagon possesses an insulinogenic action *in vivo*, as it has been shown to be the case with the administration of exogenous pancreatic glucagon, the time interrelationship of the secretion of pancreatic glucagon and insulin was investigated by determining the initial rise of the hormones following stimulation with gastrin, pancreozymin and arginine. The rise of glucagon and insulin occurred simultaneously, i.e. inside a 10 second period. We are well aware, however, that this does not exclude with certainty an insulinogenic action of pancreatic glucagon.

Glucose uptake by the pancreatic β-cells.

I.-B. Täljedal, B. Hellman, J. Sehlin. Department of Histology, University of Umeå, Umeå, Sweden.

Uptake of glucose by micro-dissected pancreatic islets of obese-hyperglycaemic mice was studied at 8°C. The use of a double-label procedure permitted correction for label in the extracellular space. L-Glucose was restricted to the sucrose space, whereas D-glucose was uniformly equilibrated over the β-cell membrane. L-glucose (5—40 mM) had no effect on the uptake of D-glucose (1 mM).

Uptake of D-glucose was saturable with V_{max} about 400 mmoles/h per kg dry islet, and with K_m around 50 mM. At a medium concentration of 5 mM D-glucose, the uptake of this sugar was almost completely blocked by 10 mM phlorizin. Under similar conditions 20 mM mannoheptulose had no significant effect on D-glucose uptake.

The results contradict the previous hypothesis that the β-cell membrane is freely permeable to D-glucose. It is suggested that uptake of glucose by these cells is mediated by a membrane-located transport molecule with stereospecificity for D-glucose. Renewed attention should therefore be given to the β-cell membrane as a possible locus for the triggering of insulin release by D-glucose.

Modification of the pancreatic β-cell function by phlorizin.

B. Hellman, Å. Lernmark, J. Sehlin, I.-B. Täljedal. Department of Histology, University of Umeå, Umeå, Sweden.

The effects of phlorizin on several parameters of β-cell function were studied with microdissected islets of obese-hyperglycaemic mice. At a concentration of 10 mM, phlorizin significantly depressed insulin release, glucose transport, glucose oxidation and the level of fructose 1.6-diphosphate, when tested in the presence of 10 mM glucose. Whereas 1 mM phlorizin inhibited glucose transport by about 50%, the glucose-stimulated insulin release remained unaffected by 1—5 mM phlorizin. In the absence of glucose, 10 mM phlorizin significantly stimulated insulin release but had no effect on the islet levels of glucose 6-phosphate or fructose 1.6-diphosphate. The results corroborate our previous hypothesis of a mediated glucose transport into the pancreatic β-cell. Insulin release does not seem to be governed by the glucose transport *in toto*. Stimulation of insulin release might rather be elicited by the binding of glucose to a distinct membrane receptor, which accounts for but a minor fraction of the total glucose uptake.

Modification of the pancreatic β-cell function by non-metabolizable, transport-specific amino acids.

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The insulin-releasing ability and uptake characteristics of non-metabolizable, transport-specific amino acids were studied *in vitro*, using microdissected pancreatic islets containing more than 90% β-cells. Among the four stereoisomers of 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH), only the b(-) form stimulated insulin release. This isomer is known as a specific substrate for transport system *L* in other cells. It was rapidly taken up by the islet cells and stimulated insulin release both in the presence and in the absence of glucose. 4-Amino-1-guanylpiperidine-4-carboxylic acid (PGA), a substrate for cationic transport systems, stimulated insulin release in the presence but not in the absence of glucose. In this respect PGA is similar to arginine. Like arginine, PGA also accumulated in the islet cells to yield distribution ratios well above unity. The results are consonant with the hypothesis that amino acids stimulate insulin release by binding to specific transport molecules.

ATP content in perifused pancreatic islets.

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A multi-channel perifusion system was used to study the dynamics of ATP regulation in microdissected islets from obese-hyperglycaemic mice. Islets were assayed for ATP at different time intervals before and after an abrupt change of the glucose concentration in the medium from 0.6 mg/ml to 3.0 mg/ml. The amounts of glucose and insulin in the effluent from the perifused islets were also recorded. At the end of a pre-incubation period (45 min), the islets from animals starved for 18 h contained about 3 mmoles of ATP per kg dry weight. This level remained constant throughout the subsequent 45-min period of stimulation with 3.0 mg/ml glucose. Similar experiments with islets from fed animals revealed a significant increase in ATP within 3 min after exposition to the high glucose concentration. In addition, the pre-incubation value was considerably higher in these islets (6.2 mmoles of ATP per kg dry weight) than in islets from starved animals. These differences in ATP between islets from fed and starved animals might, perhaps, be related to the diminished insulin response that has previously been observed after starvation.

In Vitro uptake of $^{45}Ca^{2+}$ by pancreatic β-cells.

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The possible role of Ca^{2+} in insulin secretion was investigated by studying the uptake of $^{45}Ca^{2+}$ in microdissected pancreatic islets of obese-hyperglycemic mice. The inclusion of ^{3}H-labelled mannitol in the incubation media permitted correction for extracellular and contaminating $^{45}Ca^{2+}$ without washing the islets after incubation. Exchange of Ca^{2+} was enhanced by increasing the medium concentration of glucose from 3 mM to 15 mM. However, glucose did not affect the β-cell content of $^{45}Ca^{2+}$ at equilibrium. Ca^{2+} uptake by β-cells was also stimulated by sodium deficiency. These results are consistent with a sodium-dependent mechanism for Ca^{2+} transport into the β-cells that may be of direct significance for insulin release.

Lysosomal activity and pancreatic β-cell function.

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Lysosomes might participate in secretion processes by providing enzyme activities necessary for fusion of the secretory granules with the cell membrane. The possible role of lysosomes for insulin secretion was studied in crude lysosomal fractions isolated by differential centrifugation from the β-cell-rich pancreatic islets of obese-hyperglycaemic mice. Thermal labilization at 45°C was used to release particle-bound β-glucuronidase and acid phosphatase activities. These two lysosomal marker-enzymes were assayed fluorimetrically with 4-methylumbelliferone and umbelliferone substrates. The percentile release of particle-bound β-glucuronidase after 60 min labilizaton of a crude lysosome fraction was 21.6 for islets, 51.5 for exocrine pancreas and 12.8 for liver. The higher percentage for exocrine pancreas could at least in part be attributed to the presence of a heat-labile factor, probably an enzyme. The hypoglycaemic sulphonylurea compound glibenclamide increased the release of particle-bound islet β-glucuronidase and acid phosphatase. The liberation of particle-bound islet acid phosphatase was not, however, affected by other modifiers of insulin secretion such as diazoxide, adrenaline and dibutyryl-3′.5′-cyclic AMP.

Glucose metabolism of isolated pancreatic islets of neonatal rats.

K. Asplund, C. Hellerström. Histological Department, University of Uppsala, Uppsala, Sweden.

In the foetus and newborn of several species the stimulating action of glucose on the insulin secretion is poorly developed or absent. This has been particularly well demonstrated in the rat, in which studies both *in vivo* and *in vitro* have shown a deficient insulin secretory response to glucose up to the second postnatal day.

In the present study we have examined the rates of glucose oxidation, oxygen consumption and anaerobic glycolysis in isolated islets from one and six days old rats. When incubated with 0.6 mg/ml glucose in the medium the islets from one day old rats showed a higher oxygen consumption than did those of the six days old animals. In both groups of animals, the oxidation of glucose was considerably enhanced when the extracellular glucose concentration was raised from 0.6 to 3 mg/ml. The respiratory rate was correspondingly increased only in the older animals. Without glucose in the medium the anaerobic glycolytic rate was slightly higher in islets obtained from one day old rats compared with those of the older age group. At an extracellular glucose concentration of 3.0 mg/ml the anaerobic glycolysis was more enhanced in the islets of the one day old than in those of the six days old animals.

These results indicate that the inability of the pancreatic B-cells to respond to glucose during foetal and early neonatal life should not be primarily ascribed to a deficient ability of the foetal B-cells to phosphorylate and oxidize glucose.

Quantitative measurements of enzymes involved in the turnover of fatty acids in the islets of New Zealand obese mice.

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Glucose is regarded as the main physiological stimulator of insulin release, but amino acids, fatty acids and possibly ketone bodies may also have a stimulatory effect. Little is known about the metabolism of fatty acids and ketone bodies in the B-cells. In order to evaluate the requirements for lipid metabolism of the B-cells some enzymes involved in the turnover of fatty acids have now been assayed in the islets.

Enzyme assays have been performed in frozen-dried cryostat sections by means of Lowry's microtechniques with fluorimetric detection of reduced pyridine nucleotides. In New Zealand Obese mice tissue samples have been examined from islets, exocrine pancreatic parenchyma, liver, cardiac muscle and skeletal muscle. The islets showed the highest activity of beta-hydroxyacyl-CoA dehydrogenase among the tissues studied. The B-cells thus seem enzymatically equipped for a high rate of beta-oxidation of fatty acids. ATP citrate lyase which provides cells with cytoplasmic 2-carbon fragments (in the form of acetyl-CoA) for fatty acid synthesis showed a 4—5 times higher activity in the islets than in liver and cardiac muscle. The most important pyridine nucleotide involved in fatty acid synthesis is NADPH which is formed by the hexose monophosphate shunt of the B-cells. The other enzymes which influence the NADP/NADPH ratio, glutathione reductase, NADP-dependent isocitrate dehydrogenase and malic enzyme showed considerable activities in the islets also compared with the other tissues studied. In conclusion; the islets seem enzymatically well equipped for both the degradation and formation of fatty acids. A fast turnover of the latter in the B-cells may be of importance in regulating the release of insulin and, in view of the intimate relationship between fatty

acid metabolism and glucose break-down, also in the regulation of the glucose-mediated insulin release.

Measurements of metabolic intermediates in the pancreatic islets by means of a photokinetic micro assay.

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Since the endocrine pancreas of mammals consists of numerous small islets, only minute samples can be prepared for biochemical analyses. Determinations of enzyme activities, however, are facilitated by a considerable chemical magnification, consisting in the formation of several molecules of measurable product for each molecule of the enzyme. Assay of metabolic intermediates in samples composed of a limited number of islets can be accomplished with enzymatic cycling. In this way a magnification is obtained which equals the number of cycles. Due to the possibilities of detecting low levels of light emission, applications of chemiluminescent reactions offer an alternative approach. Analytical skill in controlling cycling rates can then be replaced by quality of the electronic instrumentation. The light-producing reactions are initiated by rapid mixing of the reactants and followed by photokinetic evaluation of the response. This is accomplished by recording the time profile of the photo flash by means of a photomultiplier connected with an oscilloscope via a cathode follower.

Firefly luciferase is suitable for measurements of the ATP and ADP content of the islets. In our assay high ATP levels were found, which are in good accordance with those reported from experiments with enzymatic cycling. Bacterial luciferase is also sensitive enough for measurements at or below the picomole level which requires samples with a dry weight of about 0.1 μg. Since reduced pyridine nucleotides serve as measurable products, coupling to dehydrogenase reactions will enable a large number of analytical applications. Micro methods for assay of malate and glucose are developed and can serve as examples of direct and coupled reactions. It is also possible to measure the oxidized form of pyridine nucleotides as demonstrated by determinations of the NAD content of the islets. This has been carried out after reduction of the nucleotide with alcohol dehydrogenase.

Aspects of glucagon content and enzymatic activities in the pancreatic A_2-cells.

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Little is known about the metabolism of the A_2-cells, because of the difficulties to obtain pure samples of these cells.

In these studies sufficiant amounts of A_2-cells for microchemical analyses have been isolated by a microdissection technique with dark-field stereo-microscopy. Horses, ducks and guinea-pigs were used because the cellular distribution in their islets is favourable for isolation of A_2-cells.

The glucagon content of the A_2-cells of the horse, measured by a radioimmunological technique, was found to be in the range of 3% of their dry weight, whereas glucagon was either absent or only present in trace amounts in the B- and A_1-cells and the pancreatic acini.

The role of glucose in glucagon secretion have received much attention. Hyperglycaemia inhibits the secretory response of the A_2-cells, whereas hypoglycaemia is associated with a rise in plasma glucagon, as observed *in vivo*. In order to study the relations between glucose metabolism and the secretory process, the capacity of glucose phosphorylation of the glucagon-producing cells have been investigated, by using the Lowry microtechniques. Low hexokinase activity was observed in the A_2-cells, compared with pancreatic acini and heart muscle. The analyses were performed at high and low glucose concentration, and no high K_m hexokinase activity was found. Furthermore, the apparent K_m value for the glucose phosphorylating enzyme of the A_2-cells was 3 to 4×10^5 M. Certain enzymatic steps of the glycolysis were also studied with measurements of the activities of glyceraldehyde-phosphate dehydrogenase, pyruvate kinase and phosphoglyceric kinase. These studies indicate that the A_2-cells have a favourable enzymatic capacity for further conversion of glucose and glycolytic ATP formation.

Since the degradation of free fatty acids is of particular interest in the A_2-cells, the enzyme studies were extended to β-hydroxyacyl-CoA dehydrogenase, one of the enzymes in the β-oxidation of fatty acids. The activity of this enzyme was found to be higher in the A_2-cells than in the exocrine pancreas, but not as high as in the B-cells.

Oxidation of glucose and fatty acids in normal islets and in A_2-cell rich islets from guinea-pigs.

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It has previously been shown that there are differences in the metabolic activities of normal guinea-pig islets and guinea-pig islets containing mainly A_2-cells, which have been isolated after destruction of the B-cells with streptozotocin (Petersson, Hellerström and Gunnarsson: Horm. Metab. Res., **2**, 313, 1970). In further experiments, the production of $^{14}CO_2$ from the oxidation of ^{14}C-U-glucose and ^{14}C-1-octanoate by normal guinea-pig islets and by A_2-cell rich islets has been investigated.

It was found that the rates of ^{14}C-glucose oxidation (per μg dry weight) by normal islets and by A_2-cell rich islets were similar at 1.7 mM glucose. However, when the concentration of glucose was raised to 16.7 mM, the rate of ^{14}C-glucose oxidation by normal islets increased by 325% whereas ^{14}C-glucose oxidation by A_2-cell rich islets increased only 150% ($P < 0.01$).

The rate of $^{14}CO_2$ production from ^{14}C-octanoate was found to be higher in A_2-cell rich islets (per μg dry weight) than in normal islets ($P < 0.05$). In the A_2-cell rich islets, $^{14}CO_2$ production from ^{14}C-octanoate was higher than from ^{14}C-glucose, and raising the concentration of ^{14}C-octanoate from 0.5 mM to 5 mM caused an increase in $^{14}CO_2$ production of 600%.

It is concluded that guinea-pig islets containing a high proportion of A_2-cells oxidize octanoate more rapidly than glucose, and that octanoate oxidation by these islets responds markedly to changes in the extracellular concentration of octanoate. Octanoate also appears to be readily oxidized by the B-cells of the guinea-pig.

The correlation between the metabolism of octanoate and glucose in the A_2-cells and B-cells, and the observed effects of these compounds on glucagon release (Edwards and Taylor, Biochim. Biophys. Acta, **215**, 310, 1970) and insulin release (Montague and Taylor, Nature, **215**, 853, 1968) will be discussed.

(J.E. is in receipt of a Wellcome-Swedish Travelling Research Fellowship).

Frequency of diabetes after acute pancreatitis. Plasma insulin response to oral glucose.

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Because of the possible therapeutic implications we have felt it of importance to ascertain the frequency of diabetes after acute pancreatitis. At the same time we have studied the plasma insulin response to an oral glucose load to see whether diabetes due to acute pancreatitis is associated with a characteristic insulin response.

22 per cent of the patients had a diabetic oral glucose tolerance test. This is strikingly higher than the frequency found in the general population in the Birmingham Survey ($p < 0.01$).
In addition it was found that the blood glucose abnormality in the diabetic patients was much less than one would expect in ordinary diabetes with the same low plasma insulin responses.
The possible implications of our findings as markers to distinguish genetic from non-genetic diabetes will be discussed.

Peripheral blood flow and metabolic state in juvenile diabetics.

H.J.G. Gundersen. Second University Clinic of Internal Medicine, Kommunehospitalet, Århus, Denmark.

The relationship between 4 metabolic parameters (fasting blood sugar, FFA, St-HCO_3 and urinary glucose excretion) and blood flow in cutaneous, subcutaneous and muscular tissue in the forearm was investigated in 16 patients with classic juvenile diabetes. 7 patients had newly diagnosed diabetes and were untreated, the remaining 9 patients had diabetes for ½—5 years.
The patients were investigated both during poor and good control conditions. In all patients the blood flow in cutaneous and subcutaneous tissue was higher during poor control than in good control state. The mean blood pressure, the pulse rate, the cutaneous temperature and the body temperature were also elevated during poor control.
The total forearm blood flow, i.e. muscular blood flow, was higher during poor control in the 9 short-term diabetics. In contradistinction it was not elevated in the newly diagnosed diabetics before insulin treatment had been started.

Plasma catecholamines in juvenile diabetics

N.J. Christensen. The Second Clinic of Internal Medicine, Kommunehospitalet, Århus, Denmark.

Plasma adrenaline (A), plasma noradrenaline (NA) and total plasma catecholamine concentration (TPCA) have been measured in juvenile diabetics employing a precise and sensitive radio-enzymatic assay.
The following groups of subjects were examined in the recumbent position and 5 and 10 min after assuming the standing position:
1. 7 non-diabetic control subjects: TPCA (mainly NA) averaged 0.26 ng/ml in the recumbent position rising to 0.69 ng/ml (5 min) and 0.72 ng/ml (10 min) after assuming the standing position.
2. 9 long-term diabetics with neuropathy: TPCA averaged 0.11 ng/ml in the recumbent position rising to 0.30 ng/ml (5 min) and to 0.37 ng/ml (10 min) in the standing position. These mean values were all significantly reduced compared with the non-diabetic control subjects.
3. 6 long-term diabetics without neuropathy: values comparable with the non-diabetic control subjects.
4. 8 long-term diabetics with neuropathy hypophysectomized for diabetic retinopathy. TPCA (mainly noradrenaline) averaged 0.28 ng/ml in the recumbent position rising to 0.74 ng/ml (5 min) and 0.73 ng/ml (10 min) in the standing position. The mean values were significantly higher than in the diabetic control subjects (group 2).
The significance of elevated plasma noradrenaline levels in hypophysectomized patients for the beneficial effect of hypophysectomy on diabetic retinopathy and capillary resistance is discussed.
A number of diabetics have also been studied during keto-acidosis. Both A and NA increases considerably. It is possible that circulatory changes in keto-acidosis are partially caused by elevated plasma adrenaline levels.

Infusions of epinephrine to newly diagnosed, juvenile diabetics.

K. Arnman, S. Carlström. Med. Klin. A. Lasarettel Lund.

Male, newly diagnosed, non-insulin treated, juvenile diabetics and control subjects were given infusions of 50 micrograms epinephrine for ten minutes. The plasma concentrations of free fatty acids (FFA) and glycerol and the blood glucose concentration were followed before, during and after the infusion period. It was found that both the plasma FFA and the plasma glycerol levels rose significantly more in the diabetic patients than in the controls. The blood glucose concentration rose significantly in the control group, but concerning the diabetics no significant rise was noticed.
In earlier reports from this laboratory it have been shown that juvenile, non-insulin treated diabetics during a short period of exercise showed a higher rate of lipid mobilization than controls. In an attempt to reveal how this exercise-induced, abnormally high lipid mobilization is mediated, nor-epinephrine was infused in an earlier study into diabetics of the same type and into controls; in which study, no significant difference in lipid mobilization could be shown between the groups. The present study suggests that epinephrine may act as a mediator for the abnormally increased lipid mobilization during exercise. The mean heart rate during infusion was not different in the diabetic and the control group.
Epinephrine is known to be a stronger beta-receptor and a weaker alpha-receptor stimulant than nor-epinephrine. For that reason, the present findings may support the current theory that the lipolysis in adipose tissue in man is initiated by stimulus on the beta-receptors.

Insulin response to oral glucose tolerance test in patients with pre-eclampsia.

S.N. Madsen, I. Hindberg, L.M. Pedersen. Frederiksberg Hospital, Copenhagen.

Oral glucose tolerance tests (OGTT) with measurement of serum insulin (IRI) were performed on normal non-pregnant women ($n = 12$), on normal pregnant women ($n = 12$) and on patients with pre-eclampsia ($n = 12$). All patients were of normal weight according to the tables of Natvig.
Patients with pre-eclampsia were found to be high insulin responders after OGTT compared with the two normal groups, without impairment of the blood glucose values.

Glucose tolerance, plasma insulin and lipids in postmenopausal women during oestradiol valerate treatment.

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Eight postmenopausal women aged 41 to 53 were treated with an orally active oestrogen compound, oestradiol valerate, using a cyclical dosage schedule. Each treatment cycle consisted of 20 days of oestradiol valerate treatment at a daily dosage of 2 mg followed by a pause of 8 days. An oral glucose tolerance test (OGTT) including plasma insulin determinations was carried out before the treatment. Fasting plasma cholesterol, triglyceride and free fatty acid levels were also determined. These examinations were repeated after 3—4 months' treatment. Six women were examined also after 8—12 months' treatment.
Oestradiol valerate treatment did not cause any significant changes in blood glucose values during OGTT. Plasma insulin response tended to become slightly smaller during the treatment.
Plasma cholesterol level showed a significant decrease ($p < 0.02$) after 3—4 months' treatment and this trend persisted after 8—12 months' treatment. Oestradiol

valerate treatment did not cause any significant changes in plasma triglyceride and free fatty acid levels.

The results suggest that oestradiol valerate given to postmenopausal women at the dosage employed does not cause any unfavourable alterations in carbohydrate and lipid metabolism.

Grant: Pharmaceutical Manufacturers Leiras, Huhtamäki-yhtymä Oy, Turku, Finland.

Normal blood sugar variation during pregnancy and in the early puerperium.

A. Victor. Linköping, Sweden.

Several reports have shown that a careful management of the pregnant diabetic lowers the risk of a fatal outcome for the expected child. It seems reasonable that the goal for the management should be to keep the mothers' blood sugar values as normal as possible during pregnancy. It is known that the fasting blood sugar level decreases and the insulin response to a given glucose load increases during pregnancy.

The aim of the present investigation was to find out what is a normal blood sugar level during pregnancy. Four blood sugar values, the first after an over-night fast, were taken on 363 women in different stages of pregnancy. 21 women were followed in the first puerperial week in the same manner. The values obtained were compared with a normal material of 100 non-pregnant women of childbearing age.

It was found that the blood sugar, fasting as well as nonfasting, steadily decreases during pregnancy. The mean fasting value decreases from 76 mg% to 67 mg% with +2S-values of 91 and 84 mg% respectivly. The daily mean value decreases from a mean of 100 mg% (+2S 120 mg%) to 80 mg% (+2S 99 mg%). The +2S-value for the mean of the three nonfasting values never exceeded 150 mg%. All decreases are highly significant as tested with the Students *t*-test. In the early puerperium the blood sugar stays on the low level of late pregnancy. The reason for the lowered blood sugar level is not known, but it is probable that some factor (HPL ?) increases the sensitivity of the beta-cells in the islets of Langerhans.

From a practical standpoint it might be recommended that the blood sugar of a pregnant diabetic should never be allowed to exceed 145 mg% during the first two trimesters and in the last should be kept under 120 mg%. The daily mean value should never be let over 110 mg% and ideally should be kept about 90 mg% and in the last two months about 80 mg%.

Neonatal diabetes becoming permanent.

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A dysmature boy, b.w. 1840 g, with neonatal diabetes was presented at our Stockholm meeting in 1968. A follow up of this patient has shown, that even if the fasting blood glucose values have remained normal, and a glucose load gives a normal glucose curve, both on oral and i.v. glucose loads there are very sluggish and diminished IRI responses.

A later born sister of this patient was also dysmature, b.w. 1440 g. She showed a similar increase in blood glucose values as the brother in the neonatal period, and insulin treatment had to be started on day 12. At about one year of age we succeeded in stopping insulin for some weeks, but fasting blood glucose values increased, and tolbutamide was successfully introduced about 5 weeks later. Fasting IRI levels have always been in the low normal range. Repeated oral and i.v. glucose loading have shown diabetic glucose and a very diminished IRI-response.

Training Diabetics to Selfcontrol.

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A practical model in combination with fractionated urine-sugar analysis. The diabetic must be taught how insulin works and the mechanism of acidosis. By using a triangle with diet, insulin and physical exertion at each corner, they are taught about each factor and their interrelation. The patient should then be able to localize the cause of positive urine-tests and correct it himself, thus giving him more security and teaching him to interpret his own bodily reactions. The use of the Clinitest two-drop method and the noting of the results in a book, with explanations of the positive tests, is advocated. Routine-tests are made every fortnight for two successive days, with tests four times daily.

Metabolic effects of long-term glucagon infusion.

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8 patients with chronic congestive heart failure were given glucagon by continuous intravenous infusion for 96 h. The dose was 2 or 4 mg/h.

In all patients fasting blood sugar increased, maximal mean increase was 16 mg/100 ml. Mean fasting serum immunoreactive insulin (IRI) increased relatively more (from 9.1 to 32.0 microunits/ml), and mean fasting serum growth hormone rose from 2.8 to 11.9 ng/ml.

During glucagon infusion, glucose disappearance rate fell considerably in all patients. In spite of this, the IRI responses to the intravenous glucose were much higher than before glucagon infusions, and serum growth hormone levels increased.

Following a carbohydrate-rich meal, serum IRI responses were much higher compared with the blood sugar during glucagon infusion than before. The elevated levels of serum growth hormone were not suppressed by the carbohydrate meal when the patients were under glucagon treatment.

In conclusion, long-term infusion of large doses of glucagon leads to a state of mild diabetes mellitus in the presence of resistance to endogenous insulin. The insulin resistance may be caused by increased levels of serum growth hormone.

Latent coronary insufficiency in young diabetics.

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Graded submaximal exercise electrocardiography was performed on a bicycle ergometer by 21 young diabetics. Average age 21 years, duration of diabetes 10—20 years. In 6 ST-segment changes of the S-type were found during exercise, none had any anginal pain, or ECG changes after work. Clinical and various laboratory investigations revealed no differences between the two groups.

The results indicate that coronary disease is present in a high proportion of young diabetics before clinical manifestations appear.

Potential and latent diabetes in connection with pregnancy.

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The definitions of WHO, 1965 was used. Potential diabetic: A woman who has given birth to a baby above 4.5 kg or a stillborn with beta-cell hyperplasia. Latent diabetic: A woman with normal glucose tolerance (GT) known to have had a diabetic GT during pregnancy. However, further aspects, above all the duration of the pregnancy should be considered in connection with birth weight.

In a retrospective study (2) of 129 women who had given birth to babies above 4.5 kg intravenous glucose tolerance tests (IVGTT) were performed 10—15 years later. 10% had asymptomatic diabetes ($K < 1.0$) and 4% had developed overt diabetes. In a control group of women with normal weight children no one had $K < 1.0$. This study has now been repeated after further 5 years. In both groups mean K had decreased significantly. 24% among mothers with LD had asymptomatic diabetes while 7% among mothers to normal weight infants had developed this stage.

It would be advantageous if the diabetic stage could be revealed earlier. An IVGTT performed immediately after pregnancy is very seldom informative. However, study of the newborn overweight infant, having been exposed to a suspected decreased tolerance, might give more information. IVGTT in newborn normal infants give low K-values whereas infants of diabetic mothers show high K-values. In a study of 129 overweight infants 21% had IVGTT with high K-values (3). By analogy it is postulated that the mothers of the infants with high K-values have had a diabetic carbohydrate tolerance during pregnancy. If a gestational diabetes, which we define as a transient diabetes during pregnancy with a K-value < 1.0 in an IVGTT, is found, she is supervised in hospital. The urinary oestriol excretion is followed. Labour is induced in the 40th week. Five women who had had a gestational diabetes were followed during treatment with oral anticonception (OC) (4). IVGTT were performed during periods with and without OC. During OC there was a decreased tolerance which increased after medication was discontinued. Restraining with OC in latent diabetics is advocated.

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Perinatal foetal mortality of the Copenhagen material and on controlled therapeutical trials.

L.M. Pedersenog, J. Pedersen. Med. afd. T., Bispebjerg Hospital, Copenhagen NV, Denmark.

This contribution concerns some difficulties met with in any trial designed to show a statistically significant reduction in the perinatal mortality of infants of diabetic mothers.

The subject is illustrated with the 25 year case material of the Rigshospital centre consisting of 1164 deliveries in diabetics. The total material is divided in three periods of time, and classified according to White and the PBSP classification. All of the perinatal mortalities are given with five per cent confidence limits.

At the present time only controlled clinical trials should be acknowledged as a basis for any proposed new scheme of treatment. The difficulty lies in the small number of patients collected even in relatively big centres. Thus during the last five year period 1966—70, the perinatal mortality was 12.6% (9.0—16.2) in 318 pregnancies. In e.g. White class C the perinatal mortality was 8.2% (2.4—14.0) in 85 cases. Even without paying regard to the PBSP classification, or congenital malformations (25% of neonatal death) it would most probably take more than five years to conduct a proper controlled trial in this single White class in this centre.

Although desirable such a study is out of question. To save time it is necessary to use the total material. A trial, therefore, must be properly statistically designed beforehand, including random allocation, stratification or analysis of covariance, and perhaps sequential analysis. The help of the statistician should be requested prior to the start of a trial.

Congenital malformations in the offspring of diabetic women.

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In the centre for pregnant women with diabetes in the Royal Maternity dept. B, Rigshospitalet, Copenhagen the frequency of congenital malformations (c. m.) in the newborn infants of diabetic mothers has been consecutively and prospectively studied.

Of 1335 infants born in the period 1926 to 1971 (birth weight 1000 g or more) 8.1% had c. m. In a consecutive control series of 8789 newborn infants of non-diabetic mothers born in the same hospital in the period September 1959 to December 1961 c. m. were found in 2.8%. No difference was found in the average and distribution of maternal ages in the diabetic and the non-diabetic group. The number of c. m. in 106 infants born to diabetic mothers who came to necropsy after perinatal death in the period 1960 to 1971 was compared with that in 1734 infants of non-diabetic mothers (birth weight 1000 g or more) who were autopsied over the same period. In the diabetic group there were 37.7% and in the non-diabetic 13.7%.

In the newborn infants of diabetic mothers the over-all frequency and the frequency of major congenital malformations seem to be three to four times as high as in the general population.

As previously reported by this group the frequency of c. m. was increased in infants of mothers with vascular complications (White's classes D and F).

By comparison of the different types of malformations in the diabetic and non-diabetic materials we did not find any "specific diabetic" malformation apart from 3 cases with the combination of sacral agenesis and severe malformations of the lower limbs.

Corrigendum Notice

Diabetalogia **7**, 240—246 (1971)

G. Boden: Hormonal and Metabolic Disturbances during Acute and Subacute Myocardial Infarction in Man

Transpose legends to Figs. 1 and 2 on pages 242 and 244. Figures are correct as printed.

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