The Role of the Exocrine Pancreas in the Stimulation of Insulin Secretion by Intestinal Hormones

III. Insulin Responses to Secretin and Pancreozymin, and to Oral and Intravenous Glucose, in Patients Suffering from Chronic Insufficiency of the Exocrine Pancreas*

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Summary. In 11 non-diabetic patients with clinical and laboratory evidence of chronic exocrine pancreatic insufficiency, the effect of intestinal hormones secretin and pancreozymin upon insulin secretion, blood sugar, free fatty acids and glycerol was studied and compared with the findings obtained in 30 normal volunteers. — In the patients suffering from exocrine pancreatic insufficiency the above mentioned enterohormones did not elicit any increase in serum insulin although insulin secretion after i.v. glucose loads was perfectly undisturbed. Obviously, the mediator inducing insulin release following secretin and pancreozymin in man depends on intact exocrine pancreatic tissue. As had been expected, no differences in the serum-insulin responses to oral and intravenous glucose, as found in normals, were established in these patients. From these results it is inferred that similarity of plasma insulin changes after oral and parenteral glucose loads might hint at an early impairment of exocrine pancreatic tissue function. That implies, that a (hypothetical) "Glucose receptor" of the β -cell or its surface works more or less independently of both the exocrine pancreatic tissue and the intestinal hormones. On the other hand, the "Entero-receptor" of the β -cell responding to the insulin-stimulating action of intestinal hormones, such as secretin and pancreozymin, is likely to be more or less dependent upon sufficient amounts of intact exocrine pancreatic tissue.

Le rôle du pancréas exocrine dans la stimulation de la sécrétion d'insuline par les hormones intestinales

III. La réponse d'insuline à la sécrétine et à la pancréozymine et à des surcharges orales et intraveineuses de glucose chez des malades souffrant d'une insuffisance pancréatique exocrine

Résumé. L'effet des hormones intestinales sécrétine et pancréozymine sur la sécrétion d'insuline, sur la glycémie, les acides gras et le glycérol a été étudié chez onze malades sans diabète mais ayant une insuffisance pancréatique exocrine d'après des résultats cliniques et chimiques. Un groupe de 30 sujets normaux a été utilisé comme témoins. Les hormones intestinales n'ont causé aucune augmentation d'insuline dans le sérum des malades ayant une insuffisance pancréatique exocrine. La sécrétion d'insuline après

l'injection intraveineuse de glucose était normale. Il semble que la présence du tissu exocrine du pancréas soit nécessaire pour obtenir une stimulation de la sécrétion d'insuline par la sécrétine et la pancréozymine. Comme il était prévu, il n'y a pas eu chez ces malades — contrairement à ce qui se passe chez les personnes normales — de sécrétion d'insuline différente après l'application de glucose oral et intraveineux. Ces résultats montrent que la similitude des modifications de l'insuline plasmatique après administration de glucose par voie orale et parentérale peut signifier un mauvais fonctionnement du pancréas exocrine. On peut en déduire qu'un récepteur du glucose de la cellule bêta ou de la membrane superficielle peut opérer indépendamment du tissu pancréatique exocrine et des hormones intestinales. D'autre part, il est proposé comme conclusion qu'un «entéro-récepteur» de la cellule bêta est sensible à l'action des hormones intestinales et qu'il est dépendant, plus ou moins, d'un tissu pancréatique exocrine.

Die Rolle des exokrinen Pankreas bei der Stimulierung der Insulinsekretion durch intestinale Hormone

III. Insulinausschüttung nach Sekretin und Pankreozymmin und nach oralen und intravenösen Glucosegaben an Patienten mit chronischer Insuffizienz des endokrinen Pankreas

Zusammenfassung. Bei 11 nicht-diabetischen Patienten mit klinisch und laborchemisch nachgewiesener chronischer exkretorischen Pankreasinsuffizienz wurde die Wirkung der intestinalen Hormone Sekretin und Pankreozymmin auf die Insulinsekretion, den Blutzucker, die freien Fettsäuren und das Glycerin untersucht und verglichen mit den an 30 normalen Versuchspersonen gewonnenen Befunden. — Bei den Patienten mit exkretorischer Pankreasinsuffizienz bewirkten die oben genannten intestinalen Hormone keine Erhöhung des Seruminsulins, obwohl die Insulinsekretion nach der i.v. Verabreichung von Glucose nicht beeinträchtigt war. Offensichtlich ist für eine Insulinausschüttung nach Sekretin und Pankreozymmin beim Menschen ein intaktes exkretorisches Pankreas erforderlich. Erwartungsgemäß konnte bei diesen Patienten, im Gegensatz zu Normalpersonen, kein Unterschied in der Insulinausschüttung nach oraler und intravenöser Verabreichung von Glucose festgestellt werden. Aus diesen Ergebnissen ist zu schließen, daß die Ähnlichkeit der Plasmainsulin-Veränderungen nach oraler und parenteraler Gabe von Glucose bereits auf einen frühen Schaden der exokrinen Pankreasfunktion hinweisen könnte. Man kann daraus die Folgerung ziehen, daß ein (hypothetischer) „Glucoserezeptor“ der β -Zelle oder ihrer Ober-

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flächenmembran mehr oder weniger unabhängig von exokrinem Pankreasgewebe und intestinalen Hormonen funktioniert. Andererseits scheint der „Entero-Rezeptor“ der β -Zelle, der auf die insulinstimulierende Wirkung der intestinalen Hormone reagiert, mehr oder weniger ab-

hängig zu sein von ausreichendem Vorhandensein intakten exokrinen Pankreasgewebes.

Key-words: Secretin, pancreozymin, exocrine pancreatic insufficiency, insulin, free fatty acids, glucose.

As reported recently in this *Journal* (Hinz, Katsilambros, Schweizer, Raptis and Pfeiffer, 1971; Goberna, Fußgänger, Raptis, Telib and Pfeiffer, 1971), the insulin response of Langerhans' islets to stimulation by intestinal hormones is determined by a) the gastro-intestinal hormone applied, b) the presence or absence of glucose in the preparations *in vitro*, c) the presence of intact exocrine pancreatic tissue under both *in vitro* and *in vivo* conditions. For example, secretin did not effect insulin release from isolated islets of rat pancreas *in vitro* under any conditions examined, whereas pancreozymin did, with and without addition of glucose to the medium (Hinz, Raptis and Pfeiffer 1969; Hinz *et al.*, 1971). Moreover, in non-diabetic rats *in vivo* in which the exocrine tissue was surgically destroyed by previous ligation of the pancreatic ducts, again secretin did not evoke insulin secretion, whereas pancreozymin effected the liberation of the same amount of insulin as in the controls (Goberna, Raptis, Fußgänger, Pfeiffer, 1969; Goberna, *et al.*, 1971). These observations made under both *in vitro* and *in vivo* conditions not only confirmed the original communication of Guidoux-Grassi and Felber (1968/69) on the inactivity of secretin in releasing insulin where there was a lack of or a destruction of intact exocrine tissue of the pancreas; but they also extended this remarkable finding to the observation of the entirely different activity of other intestinal hormones (cf. Hinz, Raptis, Pfeiffer, 1969; Hinz *et al.*, 1971) which act independent of exocrine pancreatic tissue on the β -cells.

However, the question remained whether in human subjects suffering from chronic pancreatic insufficiency a similar dependence upon and an independence of the presence of intact exocrine pancreatic tissue might exist when exposing the islets to the stimulating actions of various intestinal hormones. Again, secretin and pancreozymin were selected as representatives of the two groups acting under different conditions. Furthermore, all patients under study were also subjected to i.v. and oral glucose tolerance tests. If the intestinal hormones *de facto* were involved in the impressive difference in insulin release established in normal subjects in response to oral glucose loads compared with the effects of intra-venous glucose injections, then a disappearance of that difference was expected.

Experimental Procedures and Methods

Out of 15 patients suffering from clinically proved chronic pancreatic insufficiency, 11 non-diabetic

volunteers were compared with a group of 30 metabolically healthy subjects. None of the 11 selected patients showed pancreatic calcifications by X-ray examination. The clinical course had shown several bouts of pancreatitis with elevation of amylase. The patients had steatorrhea, and in three of them laparotomy had been performed and the diagnosis of chronic pancreatitis verified. None of these patients, who had been under control for years, had an exocrine pancreatic insufficiency due to carcinoma. In all of them we found a pathological secretin-pancreozymin test, which was carried out as follows: following intubation of the duodenum and equilibration, secretin (2 units/kg body weight) (purchased from Vitrum Corporation, Stockholm, Sweden) was injected intravenously and 60 min later pancreozymin (1 unit/kg body weight) (CCK-PZ; kindly supplied by Professor Jorpes, Karolinska Institute, Stockholm) was given by the same route. For 60 min after secretin the duodenal juice was collected (the separately aspirated gastric juice discarded), and its volume and the concentration and amount of bicarbonate determined. After pancreozymin administration, we collected duodenal contents for 10 min, and determined the concentration and amount/time of α -amylase and trypsin.

The following techniques were employed: bicarbonate was measured by adding 0.5 ml samples to 1.0 ml 0.1 N HCl and back-titrating to pH 7.0 using NaOH (Preshaw and Grossman, 1965); amylase was measured by an amyloclastic method (Merckotest Amylase); trypsin was determined with N-benzoyl-arginin-p-nitroanilid (Willig and Körber, 1967); lipase with the substrate of Hauray. In several instances the tests were repeated and found to yield results that were only 10–20% at variance with the original ones.

Blood for determination of blood glucose (BG), immunologically measurable insulin (IMI), non-esterified fatty acids (NEFA) and free glycerol was taken at 5 min intervals over a period of 30 min following injections, in both the patients and the normal subjects.

On Days 1 and 2 following the gastroenterological examinations, i.v. and oral glucose tolerance tests were performed in the same subjects. On the first day a glucose load of 0.33 g glucose/kg body weight was given i.v., and on the second day 1 g glucose/kg body weight was given orally. Before, and also at times 5, 10, 20, 30, 40, and 60 min following the i.v. injection, and every 30 min up to 210 min following the oral administration, blood was taken for determinations of blood sugar, insulin, free fatty acids and glycerol. The examinations were run between 8 and 9 a.m. in supine position, after a 12–14 h fast. Time for injec-

tion of the different substances was limited constantly to 2 min. In the oral glucose tolerance tests the patients were asked to drink the glucose solution within 5 min at maximum. The blood sugar was measured in the autoanalyzer by the method of Hofmann (1937), the IMI according to Melani, Ditschuneit, Bartelt, Friedrich and Pfeiffer (1965), the NEFA by a modification of Dole and Meinertz (1960), the free glycerol by the method of Wieland (1957) in the modification of Chernick (1968).

Results

In Table 1 are given the quantitative changes in the volume and bicarbonate content of pancreatic fluid and in its content of the enzymes amylase, trypsin and lipase, induced by secretin (2 U/kg body weight) and by pancreozymin (CCK-PZ) (1 U/kg body weight)

Table 1. Changes in the volume, bicarbonate content, and enzyme content (amylase, trypsin and lipase) of pancreatic fluid following injection of secretin and pancreozymin respectively, in 30 normal human subjects and in 13 patients suffering from chronic pancreatic insufficiency (Mean $\pm$ SEM)

	n = 30 controls	n = 13 pancreatic insulin
Bicarbonate mEq	26.4 $\pm$ 2.7	9.2 $\pm$ 2.1
Volume ml/kg	4.9 $\pm$ 0.3	2.7 $\pm$ 1.0
10 min following 1 U/kg CCK-PZ I.V.		
	n = 30 controls	n = 13 pancreatic insulin
Amylase SE	65 000 $\pm$ 6 400	10 000 $\pm$ 6 700
Trypsin mU	42 000 $\pm$ 3 140	8 400 $\pm$ 1 680
Lipase mEq	459 $\pm$ 30.2	151.7 $\pm$ 31.2

Fig. 1. Blood glucose (BG) and immunologically measurable insulin (IMI) following i.v. injection of secretin and pancreozymin (CCK-PZ) in plasma of patients suffering from excretory pancreatic insufficiency ($n = 11$) and in normal controls ($n = 30$) (Mean $\pm$ SEM), resp.

respectively, in 30 normal human subjects and in 13 patients suffering from chronic insufficiency of the exocrine pancreas. Significant differences were established between the responses of the exocrine pancreatic tissues of the controls and of the patients, in all parameters measured. Simultaneously, before injection of the intestinal hormones and at 5 min intervals following the hormone administrations, i.e. during the continuous aspiration of the duodenal material by the appropriate intubation, blood was taken from the forearm vein for 30 min for determinations of blood glucose BG, IMI, NEFA and glycerol. Whereas the blood glucose values following secretin and pancreozymin either did not change or revealed only a slight but significant depression in the normal subjects, respectively, in the patients with excretory pancreatic insufficiency no differences from the initial values were

recorded; as regards the changes in the IMI levels, the brief but significant increments in the plasma insulin initiated by the intestinal hormones were found in the blood of the normal subjects exclusively, whereas the increases were completely absent in the patient group following both secretin and pancreozymin (Fig. 1).

Free fatty acids and glycerol also decreased significantly in normal subjects only after the administration of secretin and pancreozymin (Fig. 2), whereas in the patients no decreases but rather increases (of greater magnitude following pancreozymin than following secretin) were effected by the intestinal hormones.

When glucose was injected i.v., interestingly enough, no differences were found between normals and the subjects suffering from chronic pancreatic insufficiency regarding the blood glucose and IMI concen-

Fig. 2. Non-esterified fatty acids (NEFA) and free glycerol following i.v. injection of secretin and pancreozymin (CCK-PZ) in the blood of patients suffering from excretory pancreatic insufficiency ($n = 11$) and in normal controls ($n = 30$) (Mean $\pm$ SEM)

trations (Fig. 3). The K -values in the normals amounted to 1.92 ± 0.31 , and to 1.50 ± 0.49 in the patients, respectively, and the NEFA decreased accordingly, thus excluding a diabetic condition in the pancreatitis

Fig. 3. Blood glucose (BG), non-esterified fatty acids (NEFA) and immunologically measurable insulin (IMI) following i.v. administration of glucose in patients suffering from chronic insufficiency of the exocrine pancreas ($n = 11$) and in normal controls ($n = 30$) (Mean $\pm$ SEM)

group. However, clearly in contrast to these normal insulin responses of the patients to direct (parenteral) glucose stimulation, significant differences were established between controls and patients suffering from pancreatic insufficiency in the insulin responses to oral glucose loads (Fig. 4). Plasma IMI increments in the patients were significantly smaller than in the controls,

amounting to the values recorded before in this group following i.v. glucose injection, despite the fact that the blood glucose concentrations rose to higher levels and remained for a longer period elevated when compared with the controls.

In the normal subjects, the plasma insulin values actually increased from 20 ± 3 to $150 \pm 8.2 \mu$ U/ml within 30 min following 1 g glucose/kg body weight orally, whereas in the patients suffering from insufficiency of the exocrine pancreas increments from 20 ± 1.2 to only $80 \pm 4.9 \mu$ U/ml were measured; i.e., the differences in the insulin responses to oral and intravenous glucose administration when compared with those in normal subjects were lacking in the patients (Fig. 4).

Accordingly, free fatty acids and free glycerol fell more strikingly after oral administration of glucose than after i.v. injection in the normal subjects only. In the patients with chronic pancreatic insufficiency no differences were established between i.v. and oral glucose tolerance tests regarding these two parameters (Fig. 5).

Discussion

Our findings demonstrate that in patients suffering from exocrine pancreatic insufficiency due to chronic pancreatitis, neither secretin nor pancreozymin elicited the rapid release of pancreatic insulin, generally seen, and also in the controls of this study, following injection of these intestinal hormones. Furthermore, when the same patients were subjected to oral and i.v. glucose tolerance tests, the much higher increases in the blood insulin observed normally following oral glucose as opposed to i.v. glucose, were absent. On the other hand, the insulin increments in blood following i.v. glucose injection amounted to the same values as noted in the metabolically healthy controls, as did the glucose assimilation (K -values).

From these findings it might be inferred that the insulin stimulating action of the intestinal hormones is abolished when normal exocrine pancreatic tissue is absent, i.e. that the exocrine pancreatic tissue is essential for the insulin-stimulating action of the intestinal hormones to be effective. Moreover, since the specific effect of oral glucose when compared with i.v. glucose loading as regards insulin release had disappeared in the same patients, it seems very likely that in man secretin and/or pancreozymin are closely involved in the mechanism(s) mediating gastrointestinal regulation of pancreatic insulin secretion; vice versa, if the specific action of oral glucose, as opposed to i.v. glucose, is *de facto* effected via the intestinal hormones, identical insulin responses to oral and i.v. glucose should be established as an early abnormality in cases suffering from exocrine pancreatic insufficiency.

Exactly this has been observed in our cases and in the patients of Handwerger, Roth, Gorden, Sant'

Agnese, Carpenter and Peter (1969) suffering from cystic fibrosis of the pancreas. In many of their patients with "intact glucose tolerance", the reactive increases in immunoinulin in response to oral and i.v. glucose loads were the same. On the other hand, little or no elevation of serum insulin following secretin was measured by Deckert and Worning (1969) in patients suffering from severe exocrine pancreatic insufficiency

Gerard, Sescut, Paris and Linquette, 1968). Obviously, clearcut deterioration of the β -cell responses to i.v. glucose occurs only late, and then as a consequence of severe alterations of the whole pancreatic gland (Clodi and Pfeiffer, 1970).

The non-responsiveness of the Langerhans' islets located in a pancreatic gland the exocrine portion of which has been severely injured, to the insulin stimu-

Fig. 4. Blood glucose (BG) and blood insulin (IMI) concentrations in patients with excretory pancreatic insufficiency ($n = 11$) and in normal controls ($n = 30$) (Mean $\pm$ SEM) following i.v. and oral glucose loads

(Clodi, Hartmann, Rott, Raptis and Pfeiffer, 1969). Unfortunately, carbohydrate tolerance in these patients was not mentioned. Eventually, both in early and late cases suffering from calcifying pancreatitis, alterations of carbohydrate metabolism were found only in a certain percentage of the cases (Keller, Bank, Jackson, Marks and O'Reilly, 1965; Paris, Fourlinnie,

lating action of intestinal hormones, is in clear contrast to the reaction of "simple" genetic diabetics to, e.g., secretin. The same elderly diabetic subjects who respond to glucose unsatisfactorily or not at all, demonstrate insulin release following secretin and/or secretin and glucose, of the same magnitude as normal subjects (Deckert, 1968; Raptis, Schröder, Faulhaber and

Pfeiffer, 1968). The controversial findings regarding endocrine and exocrine functions of the pancreas in human diabetes mellitus hitherto reported (e.g. Chey, Shay and Shuman, 1963; Peters, Dick, Hales, Orrell and Sarner, 1966) require further studies under those new aspects.

In any case, the results of the animal experiments performed in this laboratory and published in this

studies *in vivo* on rats suffering from experimentally induced chronic insufficiency of the exocrine pancreas (Goberna *et al.*, 1969/70), and in human subjects suffering from chronic insufficiency of the exocrine pancreas due to chronic pancreatitis (this paper) or cystic fibrosis of the pancreas (Handwerger *et al.*, 1969). That implies, that a (hypothetical) "glucose receptor" of the β -cell or its surface works more or less independently

Fig. 5. Non-esterified fatty acids (NEFA) and free glycerol in patients suffering from chronic insufficiency of the exocrine pancreas ($n = 11$) and in normal controls ($n = 30$) (Mean $\pm$ SEM) following both intravenous and oral glucose tolerance tests

Journal recently (Hinz *et al.*, 1971; Goberna *et al.*, 1971) are certainly in accord with the findings obtained in the human subjects. The direct and unaffected action of glucose in stimulating insulin release from the β -cells has been demonstrated *in vitro* in studies on isolated islets stripped of all exocrine pancreatic tissue (Hinz, Raptis and Pfeiffer, 1969; Hinz *et al.*, 1971) in the

of both the exocrine pancreatic tissue and the intestinal hormones. Diabetes certainly is not the primary consequence of the deteriorations of either the gastrointestinal influences on insulin secretion or the alterations of the exocrine portion of the pancreas.

On the other hand, the (also hypothetical) "Entero-receptor" of the β -cell responding to the insulin-stimu-

lating action of intestinal hormones, e. g. secretin and pancreozymin, in man is likely to be much more dependent upon sufficient amounts of intact exocrine pancreatic tissue than in rats. Perhaps, in man that type of receptor is entirely limited to the exocrine tissues, mediating gastrointestinal control of insulin secretion solely by special intrapancreatic exocrine — endocrine channels not yet discovered.

In contrast, in rats the role of the exocrine pancreas in the stimulation of insulin secretion by intestinal hormones is more difficult to define. Both the isolated islets of Langerhans *in vitro* and the islets surrounded by degenerated exocrine pancreatic tissue do not respond to certain intestinal hormones, such as secretin, under any conditions (Guidoux-Grassi and Felber, 1968; Hinz, Raptis and Pfeiffer, 1969; Hinz *et al.*, 1971; Goberna *et al.*, 1969/70), whereas others such as pancreozymin do effect insulin release directly in all experimental preparations investigated (Hinz, Raptis and Pfeiffer, 1969; Hinz *et al.*, 1970; Goberna *et al.*, 1969/70). Therefore, the classification of the insulin stimulating actions of the intestinal hormones according to the dependence upon and the independence of intact exocrine pancreatic tissue might be justified only in certain species, e. g. rats.

References

- Chernick, S.S.: Unpublished, 1968.
- Chey, W.Y., Shay, H., Shuman, C.R.: External pancreatic secretion in diabetes mellitus. *Ann. intern. Med.* **59**, 812—821 (1963).
- Clodi, P.H., Hartmann, W., Rott, W., Raptis, S., Pfeiffer, E.F.: Verhalten des Serum-Insulins (IMI) beim Secretin-Pankrozymintest. *Verh. dtsch. Ges. Inn. Med.*, Bd. 75. München: Bergmann, J.F. 1969.
- Pfeiffer, E.F.: Mechanism of glucose intolerance in cystic fibrosis. *New Engl. J. Med.* Letter to the Editor. **282**, 455—456 (1970).
- Deckert, T.: Insulin secretion following administration of secretin in patients with diabetes mellitus. *Acta endocr. (Kbh.)* **59**, 150—157 (1968).
- Worning, H.: Insulin secretion after administration of secretin to normal and diabetic subjects and patients with chronic pancreatitis. *Diabetologia* **6**, 42 (1970).
- Dole, P.V., Meinertz, H.: Microdetermination of long chain fatty acids in plasma and tissues. *J. biol. Chem.* **235**, 2595—2603 (1960).
- Goberna, R., Raptis, S., Fußgänger, R.D., Pfeiffer, E.F.: Effect of secretin and pancreozymin on insulin secretion in exocrine pancreatic insufficiency in rat. *Diabetologia* **6**, 46 (1970).
- Fußgänger, R., Raptis, S., Telib, M., Pfeiffer, E.F.: The role of the exocrine pancreas in the stimulation of insulin secretion by intestinal hormones. II. Insulin responses to secretin and pancreozymin in experimental insufficiency on the exocrine pancreas. *Diabetologia* **6**, 68—72 (1971).
- Guidoux-Grassi, L., Felber, J.P.: Effect of secretion on insulin release by rat pancreas. *Diabetologia* **4**, 386 (1969).
- Handwerker, S., Roth, J., Gorden, P., Sant'Agnes, P.D., Carpenter, D.F., Peter, G.: Glucose intolerance in cystic fibrosis. *New Engl. J. Med.* **281**, 451—461 (1969).
- Haury, H.: Testpackung für alpha-Amylase (Diastase) im Serum und Urin. *Chem. Fabrik*, 8 München 23, Postfach 48.
- Hinz, M., Katsilambros, N., Schweizer, B., Raptis, S., Pfeiffer, E.F.: The role of the exocrine pancreas in the stimulation of insulin secretion by intestinal hormones. I. The effect of pancreozymin, secretin, gastrin and of glucagon upon insulin secretion of isolated islets of rat pancreas. *Diabetologia* **6**, 1—5, (1971).
- Raptis, S., Pfeiffer, E.F.: Comparative studies on the effects of intestinal hormones on insulin secretion by fragments of pancreas and isolated islets of Langerhans from rat. 5th Annual meeting Europ. Ass. for the study of diabetes, Montpellier 1969 (Abstr. p. 116). *Diabetologia* **6**, 49 (1970).
- Hofmann, W.S.: A rapid photoelectric method for the determination of glucose in blood and urine. *J. biol. Chem.* **120**, 51—54 (1937).
- Keller, P., Bank, S., Jackson, W.P.U., Marks, I.N., O'Reilly, I.G.: Plasma-insulin levels in "pancreatic diabetes" *Lancet* **1965 II**, 1211—1213.
- Melani, F., Ditschuneit, H., Bartelt, K.M., Friedrich, H., Pfeiffer, E.F.: Über die radioimmunologische Bestimmung von Insulin im Blut. *Klin. Wschr.* **43**, 1000—1005 (1965).
- Paris, J., Fourlinnie, J.C., Gerard, A., Lescut, S., Paris, J.C., Linquette, M.: Disturbances of glucoregulation in chronic pancreatitis. The importance of insulinemia. 14. *Franc. Mal. Appar. Dig.* **57**, 575—591 (1968).
- Peters, N.A., Dick, P., Hales, C.N., Orrell, D.H., Sarner, M.: Exocrine and endocrine pancreatic function in diabetes mellitus and chronic pancreatitis. *Gut* **7**, 277—281 (1966).
- Preshaw, R.M., Großmann, M.I.: Comparison of subcutaneous and intravenous administration of pancreatic stimulants. *Amer. J. Physiol.* **209**, 803—810 (1965).
- Raptis, S., Schröder, K.E., Faulhaber, J.D., Pfeiffer, E.F.: Stimulierung der Insulinsekretion durch Sekretin bei Diabetikern. *Dtsch. med. Wschr.* **93**, 2420—2424 (1968).
- Wieland, O.: Eine enzymatische Methode zur Bestimmung von Glycerin. *Biochem. Z.* **324**, 313—319 (1957).
- Willig, F., Körber, W.: Eine Methode zur Bestimmung Trypsin und Chymotrypsin im Stuhl mit Aminosäure p-nitroaniliden. *Z. Gastroenterologie* **5**, 33—36 (1967).

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