Diabetologia 9, 217–231 (1973)

Biochemical and Morphological Investigations of 30 Human Insulinomas

Correlation between the Tumour Content of Insulin and Proinsulin-Like Components and the Histological and Ultrastructural Appearance

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Received: October 15, 1972, accepted: January 3, 1973

Summary. Thirty human insulinomas have been investigated histologically and their immunoreactive insulin (IRI) content estimated. In most cases immunohistological and ultrastructural studies were also performed and the percentage of proinsulin-like components (PLC) in the tumour determined. Except for 1 case the IRI concentration in the tumours was lower (0.01–89.0 U/g) than in the islet tissue. Histologically, immunohistologically and ultrastructurally a variable number of tumour cells contained few and often no beta-granules, indicating a decreased storage capacity for insulin. This defective storage capacity seems to be the major functional abnormality of insulinoma cells. Ultrastructurally four types of insulinoma can be distinguished. The ultrastructural diagnosis of an insulinoma can only be made in type I (typical beta-granules, 13 cases) and type II (typical and atypical granules, 7 cases) but not in type III (atypical granules only, 4 cases) and type IV (virtually agranular, 4 cases). The type IV tumours had the lowest IRI concentration and did not respond to diazoxide treatment. The IRI concentration of the uninvolved pancreas of 19 patients was 2.0 ± 0.2 U/g and in the range of non-diabetic adults. — The percentage PLC in 19 insulinomas was higher (5.3–22%) than in the pancreas of human adults with and without insulinoma (1.7–4.8%). The percentage of PLC in the serum of patients with insulinoma was always higher than in their tumours (33–61%). It is suggested that the higher PLC levels found in the tumour and serum of insulinoma patients are the consequence of the reduced storage capacity of the tumour cells resulting in a rapid passage through the granular route or even a non-granular release of newly synthesized insulin.

Key words: Insulinoma, IRI content of insulinoma, ultrastructural categorization of insulinomas, proinsulin content of insulinomas, functional defect in insulinomas, reduced storage capacity of insulinoma cells, non-granular insulin release, proinsulin content of human pancreas, diazoxide response of insulinomas.

The clinical syndrome hypoglycaemia due to insulin-producing tumours of the pancreas is fairly uniform and the criteria for diagnosis are well established. Insulin has been extracted in several series of functioning pancreatic adenomas and carcinomas [12, 40, 52]. These reports have demonstrated that generally the cells of insulinomas[1] contain less insulin than normal B-cells. Therefore, it has been concluded that hyperinsulinism in patients with an insulinoma is not due to an excessive production of insulin but to an inability to control its storage and release [12, 34]. Reports about ultrastructural investigations of insulinomas are usually confined to single or few cases [3, 14, 20, 21, 25, 26, 28, 41] and, therefore, do not give sufficient information about the broad scale from nearly normal B-cells [3, 21, 25, 26, 28] to atypical cells [3, 14, 20] and the frequency in which they occur in different insulinomas. Of special interest is the correlation of the histology and ultrastructure of insulinomas with the insulin content of the tissue; this information may be relevant for the suggested defect in insulin storage and release and may explain the different response of patients with insulinomas to agents which block insulin release, like diazoxide. It has been claimed that this drug is effective only in patients with insulinomas which store insulin in granular form, while virtually agranular tumours do not respond to diazoxide treatment [8, 9, 10].

Since the discovery of proinsulin by incubating slices of insulinoma tissue *in vitro* [51] and that this material could be detected in the circulation [43, 44], different studies have demonstrated that patients with insulinomas have a higher percentage of proinsulin or proinsulin-like components in their serum than normal persons [16, 17, 18, 22, 36, 49]. Again, it has been suggested that this phenomenon may be due to a defective storage mechanism of insulin [19, 36, 45]. However, from only 3 single cases has the proinsulin content of insulinomas been reported; in 2 it was elevated [18, 36] and in 1 within the normal range [29]. These data were not correlated with the ultrastructurcal appearance of the tumours.

This study presents data collected from a series of 30 human insulinomas and correlates their insulin and proinsulin content with their histological and ultrastructural appearance. By comparing these data with the findings in the unaffected pancreas of the same

1 The expression "insulinoma" is used throughout this article instead of "insulin-producing islet tumour" or "insulin-producing insulinoma" or "insulin-producing pancreatic tumour". The terminology stresses the fact that the main characteristic of these tumours is their ability to produce and secrete insulin while their morphological appearance may vary and their origin is not yet defined.

cases a better understanding was sought of the mechanism of storage and release of insulin and its precursors in man under normal and pathological conditions.

Material and Methods

Thirty insulinomas (28 adenomas and 2 carcinomas) were collected during the last 7 years. All tumours have been obtained in the operation theatre immediately after extirpation. The tumour was measured (for evaluation of the weight) and divided with razor blades for histological and biochemical investigations. To avoid tissue damage during the extirpation a small piece of the tumour was excised before clamping off the vessels and fixed immediately for ultrastructural studies. In most cases normal pancreatic tissue was attached to the extirpated tumour or partial pancreatectomy was necessary. Thus biochemical and morphological investigations could be performed in the pancreas of 21 cases. For control pancreatic tissue of non-diabetic patients (suspected or proven Zollinger-Ellison syndrome) was obtained during surgery. Pancreatic tissue of 3 patients was studied biochemically and morphologically and of 5 patients only morphologically.

Determination and fractionation of IRI in serum and tissue

IRI in serum was determined by a slightly modified standard procedure [35] using anti-porcine guinea pig serum, ^{125}I-porcine insulin (Hoechst), and crystalline human insulin (Novo Research Institute) as reference standard.

Tissue from pancreas and tumours (300 to 800 mg) was extracted twice with 5 ml of acid-ethanol (375:105:7.5; ethanol: H_2O:HCl). The serum (50 to 75 ml) was extracted once with 10 vol. of acid-ethanol (375:7.5; ethanol:HCl). Aliquots of the tissue extract were neutralized and assayed for IRI by a back titration procedure [33] using anti-porcine guinea pig serum and ^{125}I-porcine insulin. The acid-ethanol extracts of the tumour and pancreas tissue and of serum were chromatographed on Sephadex LH-20 (tissue extracts: 2.5×20 cm; serum extracts: 8×30 cm) with 1 N acetic acid. In both cases the void volume was pooled, lyophilized and rechromatographed on Sephadex G-50 fine (2.0×95 cm) in 1 N acetic acid/0.15 M NaCl. Successive 1 ml fractions were assayed for IRI [35]; when samples had to be assayed at low dilutions, 0.5 M phosphate-buffer (pH 7.4) was used instead of 0.1 M borate-buffer.

Calculation of percentage of proinsulin-like component (PLC)

By chromatography of the extracts on Sephadex G-50, two major peaks of IRI were detected. The first peak of several tumour extracts was exposed to limited tryptic hydrolysis yielding IRI-material eluting in the position of the second peak, insulin. Thus, the first peak contained PCL and the second insulin. The IRI in the PLC (peak I) was expressed as a percentage of the total IRI (peak I plus peak II). Crystalline human insulin[2] served as the reference standard in these assays. Bovine proinsulin[2] was tested in the assay system to determine its affinity to the antibody (the same lot of guinea pig anti-insulin serum being used throughout all of these experiments). Compared with equimolar quantities of human insulin, the bovine proinsulin was about 50% less active in displacing ^{125}I-insulin (Fig. 1). No correction for this underestimation of PLC was made.

2 Gift from Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen.

Histology

Tissue samples of the tumours and the adjacent pancreas were fixed in Bouin's fluid and embedded in paraffin. Thin sections were stained with hematoxylin eosin and aldehyde-thionin [38]. Immunohistology was performed as described previously [1]. The amount of connective tissue in the tumour was roughly estimated and the aldehyde-thionin stained sections were evaluated for the percentage of stained and unstained endocrine cells and the intensity of the staining reactions in the stained B-cells.

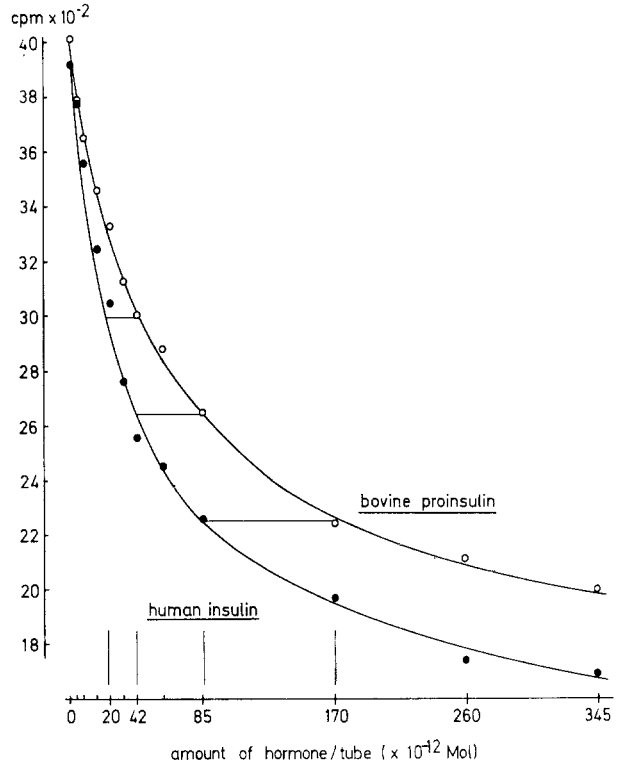

Fig. 1. Displacement of ^{125}I-porcine insulin (0.1 ng/tube) from an anti-porcine insulin serum by human insulin (●——●) or bovine proinsulin (○——○). The antiserum has about a 50% lower affinity for bovine proinsulin than human insulin on a molar basis

Electron microscopy

For electron microscopy the tissue was immediately fixed by immersion in Karnovsky's fixative buffered with 0.1 M sodium cacodylate with 0.03% calcium chloride. After 3 h the tissues were washed overnight in the same buffer and transferred to 2% osmium tetroxide in sodium-cacodylate buffer. After rinsing, the tissue was dehydrated in a graded series of acetone and embedded in vestopal. Ultra-thin sections were cut on an LKB ultramicrotome, picked up on formvar-coated copper grids and stained with uranyl acetate and lead citrate [6]. The sections were viewed in the Zeiss electron microscope EM 9 S with a built-in condenser.

In the electron micrographs (frequently using photomontage for reconstruction of larger areas) the following findings were evaluated: The frequency and the type of secretory granules in comparison with normal B-cells of human islets of Langerhans and the development of the rough endoplasmic reticulum and number of small cytoplasmic vesicles depicting the degree of functional activity.

Results

All individual data of the investigated cases relevant to this study are given in Table 1. Here, the cases are listed according to the date of operation. The case numbers of Table 1 are used throughout the text and the figures.

1. Clinical findings

The clinical findings in these 30 patients may be briefly summarized as follows. 80% showed, after an overnight, fast blood glucose levels below 50 mg/100 ml and 90% serum insulin (IRI) levels above 50 μU/ml (if investigated frequently). Excessive serum insulin concentrations were found in 80% of the patients after challenge with glucose, tolbutamide or glucagon. There were no correlations between the insulin concentration or content of the tumours and the IRI release after glucose, tolbutamide or glucagon stimulation [cf. 12, 40]. The best test for establishing the diagnosis was prolonged fasting (up to 36 h): All patients showed, after prolonged fasting, hypoglycaemic symptoms accompanied by low glucose levels and responded promptly to glucose injection. Twenty four patients were treated with diazoxide preoperatively. Twenty responded well, while 4 did not respond (2 patients with B-cell adenoma and 2 patients with B-cell carcinoma). The observations during treatment with diazoxide in some of the cases have been described and discussed previously [8, 13].

2. Insulin concentration in tumours and pancreas

The IRI concentration has been estimated in all 30 tumours. It ranged from 0.01 to 199.0 U/g wet tissue (mean 23.8 ± 5.0) (Fig. 2). The 2 carcinomas had the lowest insulin concentration (0.01 and 0.2 U/g). These 2 cases and 2 further patients with the next lowest insulin concentration in their tumours (0.75 and 1.1 U/g) did not respond to diazoxide.

The IRI concentration in the pancreas has been estimated in 21 cases (Fig. 2). It ranged from 0.49 to 4.85 U/g wet weight (mean $= 2.0 \pm 0.2$). The insulin concentrations in the pancreas of 3 non-diabetic patients without an insulinoma were in the same range (0.77, 1.2 and 1.9 U/g). Assuming an islet volume of 1 per cent of the pancreas, only in 1 out of 30 insulinomas (case No. 2) did the tumour cells contain as much

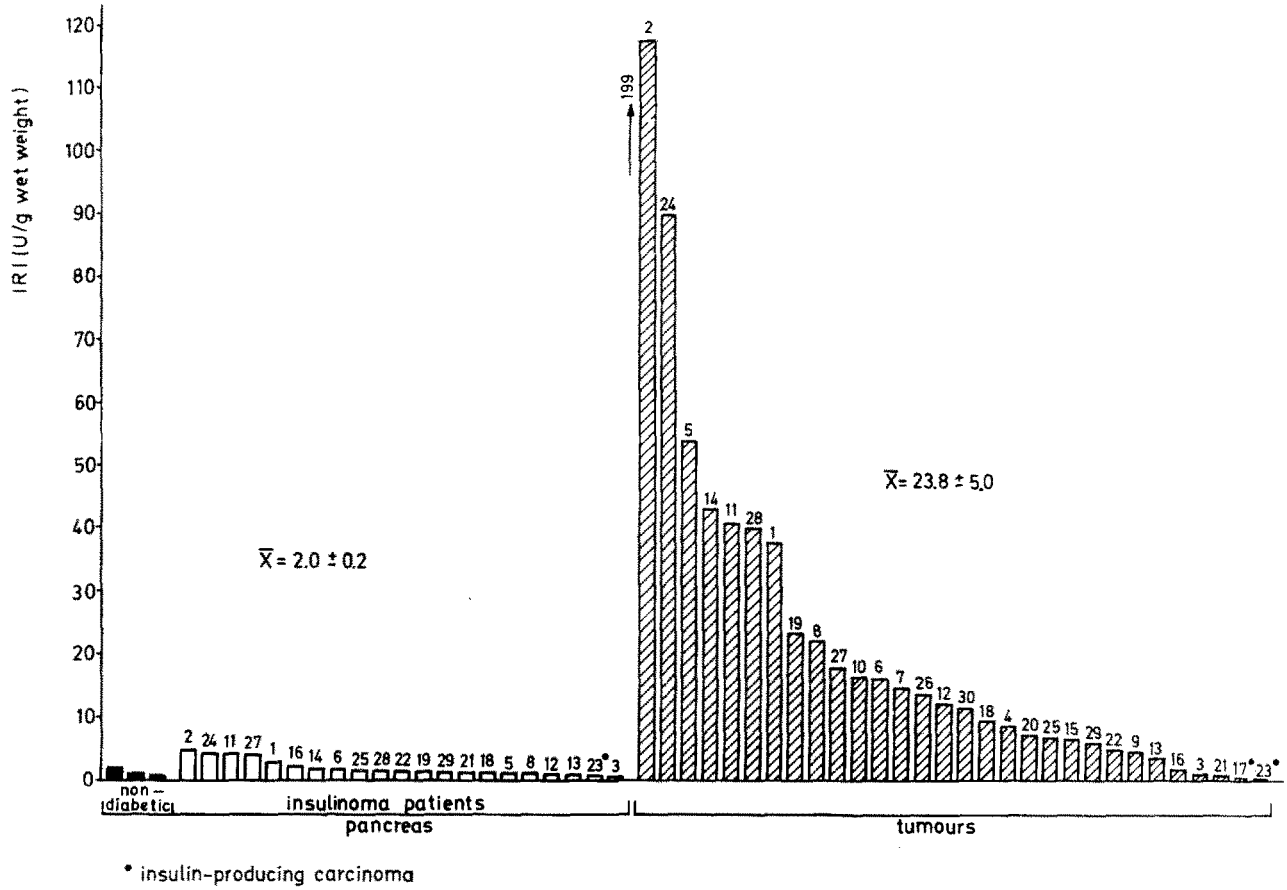

Fig. 2. Concentration of immunoreactive insulin (IRI) in the pancreas of 3 non-diabetics (black columns) and of 21 insulinoma patients (open columns) and in 30 insulinomas (hatched columns). Two cases with carcinoma are marked (●). The case numbers (see Table 1) are written at the top of each column

insulin as the normal B-cells; the majority of the tumours contained much less insulin per cell.

Fig. 3 gives the total insulin content (mean 82 ± 12 U) of the tumours and the pancreatic glands, assuming the average weight of the pancreas to be 100 g. Only 2 cases contained more insulin in the tumour than the mean value of all pancreatic glands (mean 200 ± 20 U); none more than the pancreas with the highest insulin content. Even by adding the insulin content of the tumour and the pancreas for each case total IRI concentration are listed in Fig. 4. The PLC ranged in the pancreas of patients with insulinomas from 1.7 to 4.8% (mean 2.5 ± 0.2). The values in the pancreas from non-diabetic patients without insulinoma were in the same range (2.0, 2.0 and 3.0%). The PLC values in the insulinomas ranged from 5.3 to 22.0% (mean 13.8 ± 0.8). Thus, they were significantly higher ($p < 0.01$) than in the pancreas. A nearly normal value was found in case No. 24. This case had the highest IRI concentration (89.0 U/g) of all tumours in

Table 1

Case No.	Name and place of operation		Sex	Age	Adenoma or carcinoma	Connective tissue[b]	Aldehyde-thionin stain		Ultrastructural granular type[c]
							% of stained cells[b]	intensity of reaction	
1	Stu.	Gö	♀	62	ad	+	+++	++	–
2	Schau.	Gö	♀	58	ad	+	+++	+++	–
3	Schra.	Gö	♀	55	ad	○	○	○	IV
4	Elb.	Gö	♂	67	ad	+++	+++	++	I
5	Reit.	M	♂	57	ad	+	++	++	I
6	Böhl.	Gö	♂	37	ad	+	○	○	III
7	Mey.	Gö	♀	69	ad	+++	++	+++	I
8	Kor.	M	♀	67	ad	++	++	+	I
9	Kalt.	Fr	♀	45	ad	+	+	+	I
10	Schul.	M	♂	39	ad	○	+	+	III
11	Lep.	Gö	♀	46	ad	+++	+++	++	I
12	Müll.	Gö	♀	28	ad	+	+++	+	I
13	Wall.	Gö	♀	37	ad	+	+	+	I
14	Bi.	M	♀	23	ad	+++	+++	+++	II
15	Lang	M	♀	63	ad	○	(+)	+	II
16	Frau.	M	♀	74	ad	++	○	○	III
17	Rom.	Gö[a]	♀	34	ca	++	○	○	IV
18	Bal.	Gö	♀	52	ad	+	+	+	II
19	Schaa.	Gö	♀	63	ad	+	++	+	II
20	Krau.	Wü	♀	65	ad	++	+	++	II
21	Ott.	Be	♂	51	ad	+	○	○	IV
22	Pet.	Gö[a]	♂	38	ad	++	+	+	II
23	Walt.	Mz	♀	51	ca	+	○	○	IV
24	Bök.	Gö	♀	43	ad	○	+++	+	I
25	Zitz.	N	♀	24	ad	+	○	○	III
26	Ben.	Gö	♀	45	ad	+++	++	++	I
27	Mur.	Gö[a]	♂	40	ad	+	+	+	I
28	Schal.	Be	♂	64	ad	○	++	++	II
29	Kön	Be	♂	59	ad	○	(+)	+	I
30	Kle.	Gö[a]	♀	28	ad	+++	++	++	I

[a] transferred from Homburg (Saar)
[b] ○ = none; (+) = minimal; + = <20%; ++ = 20–50%; +++ = >50%
[c] ultrastructural categorization see text
[d] estimation of metastases seen during operation
[e] estimated assuming 100 g pancreas weight
[f] partial pancreatectomy

this sum surpasses only in four cases the highest insulin content of the pancreas. Only in 11 cases did the total insulin amount stored in the tumour and the pancreas exceed the mean insulin content of the normal pancreas.

3. Proinsulin-like components (PLC) in tumours, pancreas and serum

The percentage PLC was estimated in 19 tumours, in 9 pancreatic glands of insulinoma patients and 3 non-diabetic patients. The results as percentage of which the PLC were estimated. The highest PLC (22%) was found in 1 carcinoma (case No. 23) while the other carcinoma contained PLC near the mean value of all tumours (14%).

Generally, the highest percentage of PLC was found in the tumours with the lowest IRI concentration. However, this correlation was not significant ($r = 0.579$) even if the IRI concentration was corrected according to the different content of connective tissue in the tumours.

The PLC content of the serum was estimated in

6 cases during glucose load. The levels were much higher than in the tumour extracts (33—61%). Fig. 5 gives an example of the concentration pattern of PLC in the tumour and the serum of case No. 17.

4. Histological findings

All 28 insulinomas were investigated histologically. The 26 adenomas consisted of islet-like cells which formed either solid, ribbon-like or acinar arrangements. Mitoses were observed extremely rarely. In some tumours ductular structures were present in between the endocrine cells. However, most tumours had close connection to hyperplastic ductular structures, from which endocrine cells budded out, or contained ductular elements. In 2 cases round colloid bodies were found in many tumour cells, similar to those described in single insulinomas [2, 11, 20, 21). The 2 carcinomas were characterized by a greater variety of the nuclear size of their cells and the more frequent occurrence of mitoses.

The amount of connective tissue separating the cords and nests of endocrine cells varied considerably between the tumours. Since this is of relevance for the interpretation of the insulin concentration in the tumours, the percentage of connective tissue was roughly estimated in each case. The results are listed in Table 1. Some tumours contained amyloid. However, this finding will be discussed in a separate publication.

In the aldehyde-thionin stained sections only few tumours showed a very strong staining reaction similar to normal human islets of Langerhans. More frequently, only the capillary pole of the tumour cells reacted positively. In addition, many tumours showed a variable number of tumour cells which were not stained with aldehyde-thionin at all, suggesting that a number of tumour cells only store small amounts of insulin. The percentage of positively stained cells is given separately from the average staining intensity of the positive cells in Table 1.

From these data it can be tentatively concluded that the aldehyde-thionin stain is most intensive in the tumours with the highest IRI concentration, especially

Table 1

Tumour weight g	Extractable IRI U/g tumour	Extractable IRI U/g pancreas	PLC as % of IRI tumour	PLC as % of IRI pancreas	IRI content U tumour	IRI content U pancreas[e]	PLC as % of IRI in serum	Diazoxide response
12.0	37.8	2.7	—	—	453.6	270.0	—	+
0.9	199.0	4.9	—	—	179.1	490.0	—	+
5.5	1.1	0.5	21.0	—	6.1	50.0	—	○
4.5	8.6	—	—	—	38.7	—	—	+
3.0	54.2	1.2	—	—	162.6	120.0	—	+
2.5	16.1	1.8	—	—	40.3	180.0	—	+
5.0	14.9	—	—	—	74.5	—	—	+
3.0	22.4	1.2	—	—	67.2	120.0	—	+
5.0	4.7	—	—	—	23.5	—	—	+
8.0	16.3	—	15.0	—	130.4	—	—	+
3.0	40.9	4.3	—	—	122.7	430.0	—	—
2.0	12.2	1.0	—	—	24.4	100.0	—	+
4.0	3.6	0.9	—	—	14.4	90.0	—	+
2.5	43.2	1.9	13.0	—	108.0	190.0	—	+
3.5	6.6	—	11.0	—	23.1	—	—	+
25.0	1.7	2.3	21.0	—	42.5	230.0	—	+
> 500.0[d]	0.2	—	14.0	—	100.0	—	61.0	○
18.0	9.4	1.2	10.0	—	169.2	120.0	—	+
1.0	23.5	1.5	12.6	4.8	23.5	150.0	42.0	+
12.0	7.3	—	10.6	—	87.6	—	—	—
4.0	0.8	1.4	18.5	1.9	3.2	140.0	—	○
15.0	4.8	1.5	19.3	2.4	72.0	150.0	36.0	+
> 500.0[d]	0.01	0.8	22.0	3.2	5.0	40.0[f]	—	○
3.0	89.0	4.4	5.3	1.7	267.0	440.0	33.0	+
3.0	6.9	1.7	15.3	2.8	20.7	170.0	—	—
5.0	13.6	—	8.5	—	68.0	—	—	—
3.0	17.9	4.1	7.3	2.3	53.7	410.0	38.0	—
1.0	40.1	1.6	14.0	1.8	40.1	160.0	47.0	+
1.4	6.0	1.4	9.5	1.8	8.4	140.0	—	—
2.0	11.5	—	14.2	—	23.0	—	—	+

considering the amount of connective tissue present in the tumour. The same general conclusion can be reached regarding the percentage of PLC. This is highest in the aldehyde-thionin negative tumours and only slightly elevated in those insulinomas in which the majority of the cells react strongly (Table 1). Thus, the less unstained cells are present in an insulinoma, the lower the percentage of PLC seems to be in the tumour extract. Some exceptions to this general pattern have been observed in tumours with insulin concentrations from 5—10 U/g and, despite, this showing virtually no cases the cells sometimes differed considerably in different areas of the tumour. The majority of the tumours contained cells with secretory granules as seen in normal B-cells of human islets of Langerhans. These were of two types: 1. electron-dense (multishaped or round) and surrounded by a membranous sac encompassing an electron lucent space; 2. pale and round with only a small or no space between the granule and the limiting membrane (Fig. 6). The frequency of these different granule types varied as it does in the normal B-cells and showed no correlation with either the in-

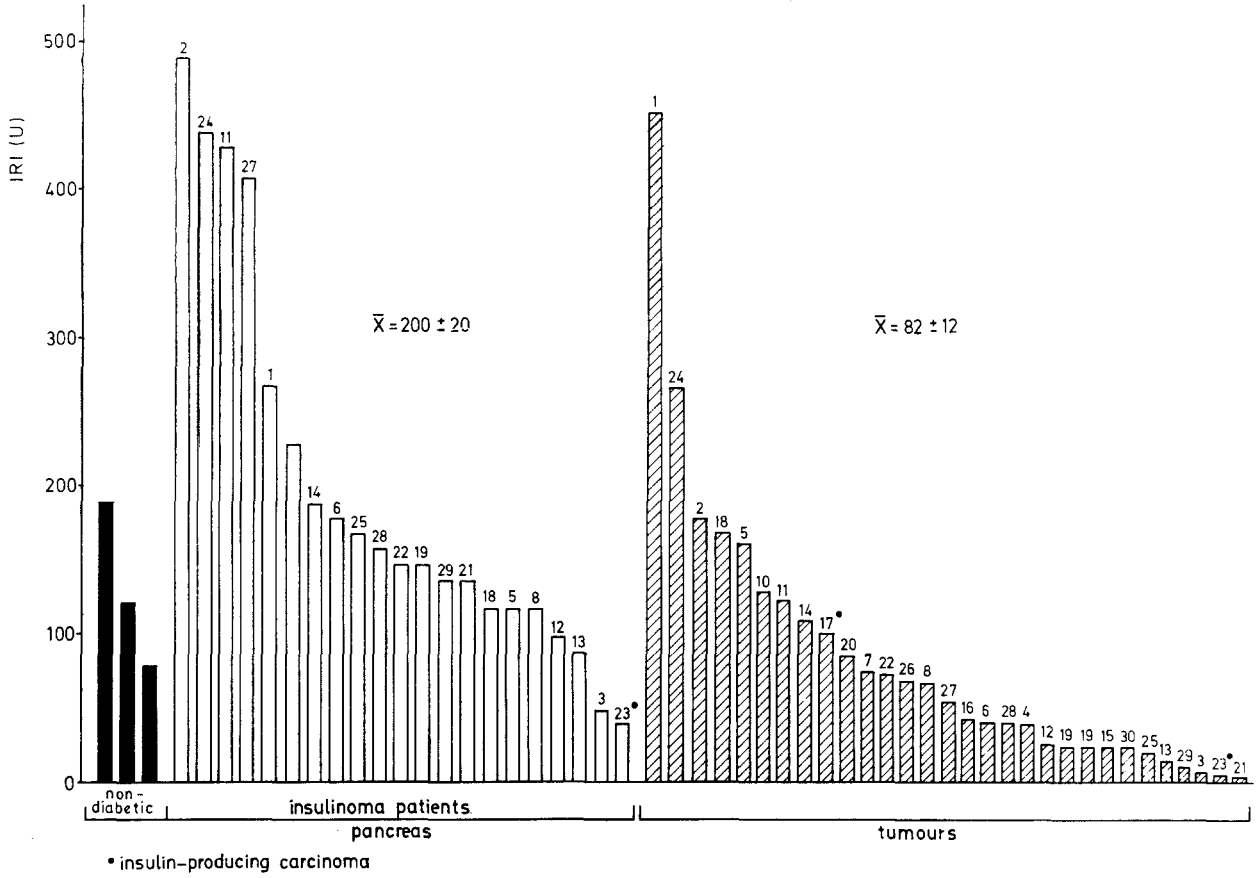

Fig. 3. Total IRI content of the pancreas of 3 non-diabetics and 21 insulinoma patients and of 30 insulinomas. For details see Fig. 2

aldehyde-thionin positive cells. At the same time the immunohistological reaction for insulin was strongly positive in many cells. However, this reaction is, according to published findings [1], mainly a qualitative one, indicating the presence but not the amount of insulin in the cells as the aldehyde-thionin stain does. Only in tumours with insulin concentrations below 1.0 U/g was the immunohistological reaction negative.

5. *Ultrastructural findings*

Twenty eight of the 30 insulinomas were investigated ultrastructurally. The ultrastructural appearance of the tumours was not uniform. Even in individual sulin content or PLC content of the tumours. In addition to these typical beta-granules, atypical granules occurred either in the same cells or in different cells of the same tumour (Fig. 7). Few tumours revealed only cells containing such atypical secretory granules. These atypical secretory granules were electron-dense and round, usually smaller than normal beta-granules and had only tightly fitting or no discernible membranes (Fig. 8). Such granules can be seen also in other endocrine tumours and do not allow the diagnosis of an insulinoma. Since tumours with only atypical secretory granules often showed a strongly positive immunohistological reaction and contained appreciable

amounts of IRI, it is concluded that insulin can be stored in this abnormal form in some tumours. The aldehyde-thionin stain was mostly negative in tumours containing only atypical granules despite IRI concentrations ranging from 2.3 to 16.3 U/g.

Beside tumour cells containing typical or atypical secretory granules some cells in each tumour were only poorly granulated or virtually agranular (Fig. 9). These cells often showed signs of high functional activity with well developed rough endoplasmic reticulum and Golgi zone and numerous cytoplasmic vesicles and, in addition, many lysosomes. Few tumours (including the 2 carcinomas) had virtually only agranular cells which revealed all signs of high functional activity (Fig. 10). Their insulin content was extremely low (0.01 to 1.1 U/g) and the patients did not respond clinically to diazoxide treatment. They also contained high levels of PLC. Other islet cells (A-cells and D-cells) were not found with certainty in the tumours. However, in many tumours single cells were observed which resembled the enterochromaffin cells (EC-cells) found in the gastrointestinal mucosa and in carcinoid tumours. These EC-cells were most frequent in the two carcinomas (Fig. 11).

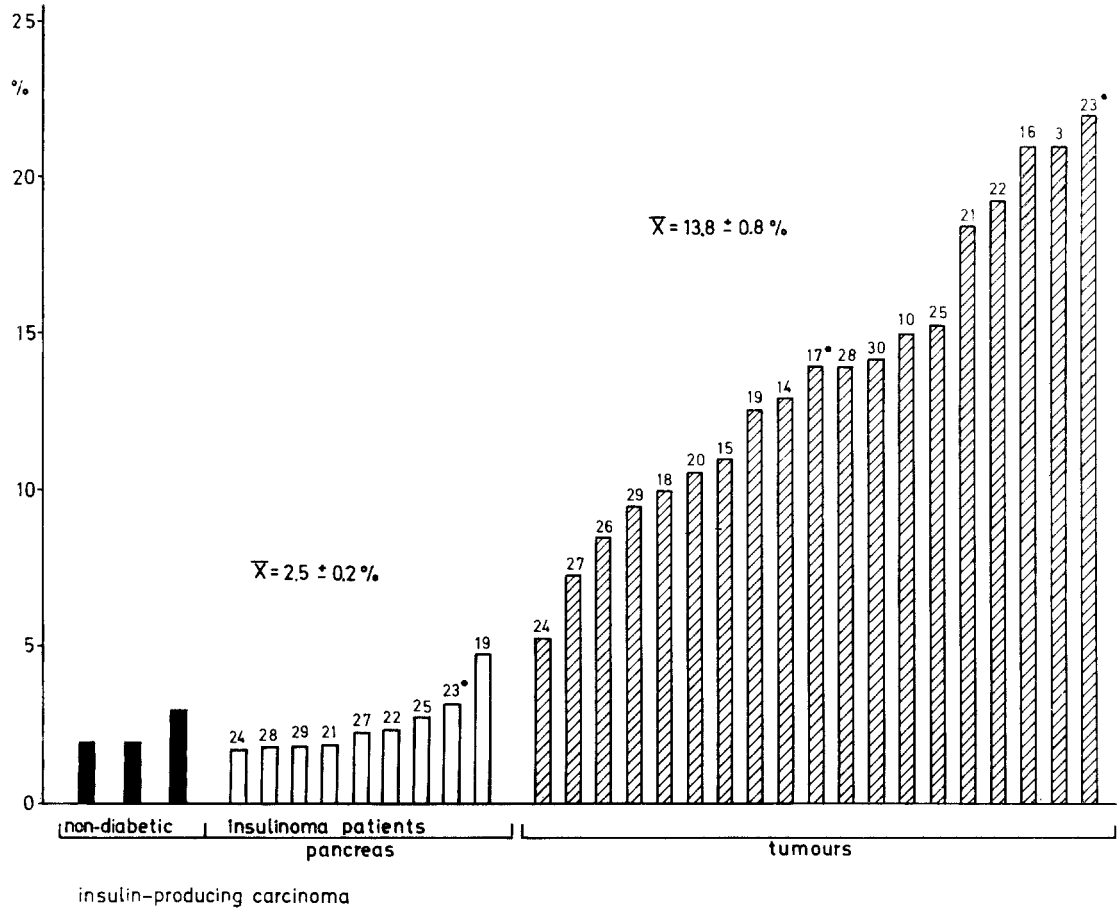

Fig. 4. Proinsulin-like components (PLC) as a percentage of the IRI concentration in the pancreas of 3 non-diabetics and 9 insulinoma patients and in 19 insulinomas. For details see Fig. 2

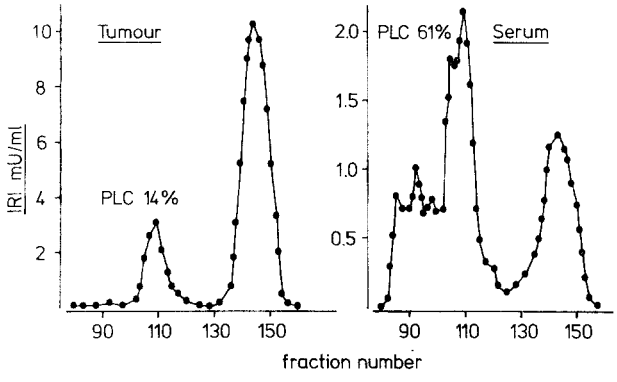

Fig. 5. Sephadex G-50 gel filtration patterns of tumour and serum extracts from a patient with an insulin-producing carcinoma of the pancreas (Case No. 17, Table 1)

According to their ultrastructure, the 28 insulinomas can be categorized into the following four types:

I. Tumours with cells containing secretory granules typical for human islet B-cells (Fig. 6).

II. Tumours with cells containing typical and atypical secretory granules (Fig. 7).

III. Tumours with cells containing only atypical secretory granules (Fig 8).

IV. Tumours which only contain virtually agranular cells (Fig. 10).

addition, the IRI concentration and the percentage of PLC is listed. From this it can be concluded that in the type I tumours, which are most frequent, the IRI concentration is highest and PLC content least ele-

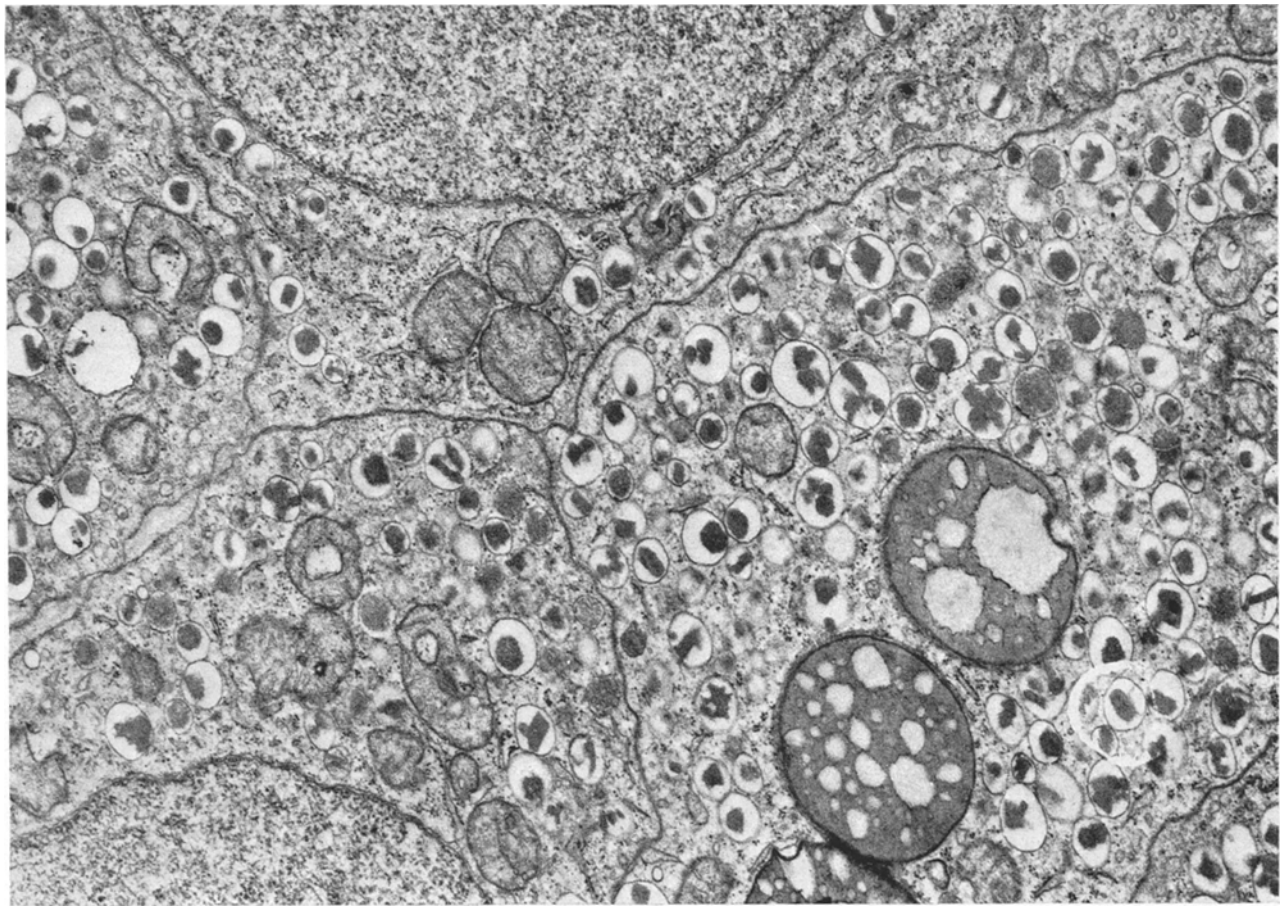

Fig. 6. Adjacent portions of 5 insulinoma cells showing typical beta-granules (Type I insulinoma). Crystalline, round and amorphous cores surrounded by an electron lucent space encompassed by a limiting membrane. One cell contains large lipid bodies. (Case No. 7, Table 1) ×24000

Table 2. *Categorization of 28 insulinomas according to the ultrastructural type of secretory granules*

	Number of cases	Mean IRI concentration U/g (range)	Mean PLC as % of IRI (range)	Stainable with aldehyde-thionin	Immunohistological reaction for insulin
I. tumours with typical β-granules	13	24.1 (3.6—88.9)	9.0 [5][a] (5.3—14.2)	+	+
II. tumours with typical and atypical β-granules	7	19.3 (4.8—43.2)	12.9 [7][a] (10.6—19.3)	+	+
III. tumours with atypical granules only	4	10.2 (1.7—16.3)	17.1 [3][a] (15.0—21)	0	+
IV. virtually agranular tumours	4	0.5 (0.01—1.1)	18.8 [4][a] (14 —22)	0	0

[][a] Number of cases investigated for PLC.

In type III and IV the diagnosis of an insulinoma is not possible on ultrastructural grounds and with granule stains. Table 2 gives the frequency with which the different ultrastructural types were found. In

vated. The type IV tumours have the lowest insulin concentration and highly elevated PLC levels. Type II and III are in between both these extremes. A correlation between ultrastructural findings and IRI and

PLC concentration is more evident if the number of agranular cells is also accounted for: the more virtually agranular cells in a tumour, the lower the IRI concentration and the higher the percentage of PLC.

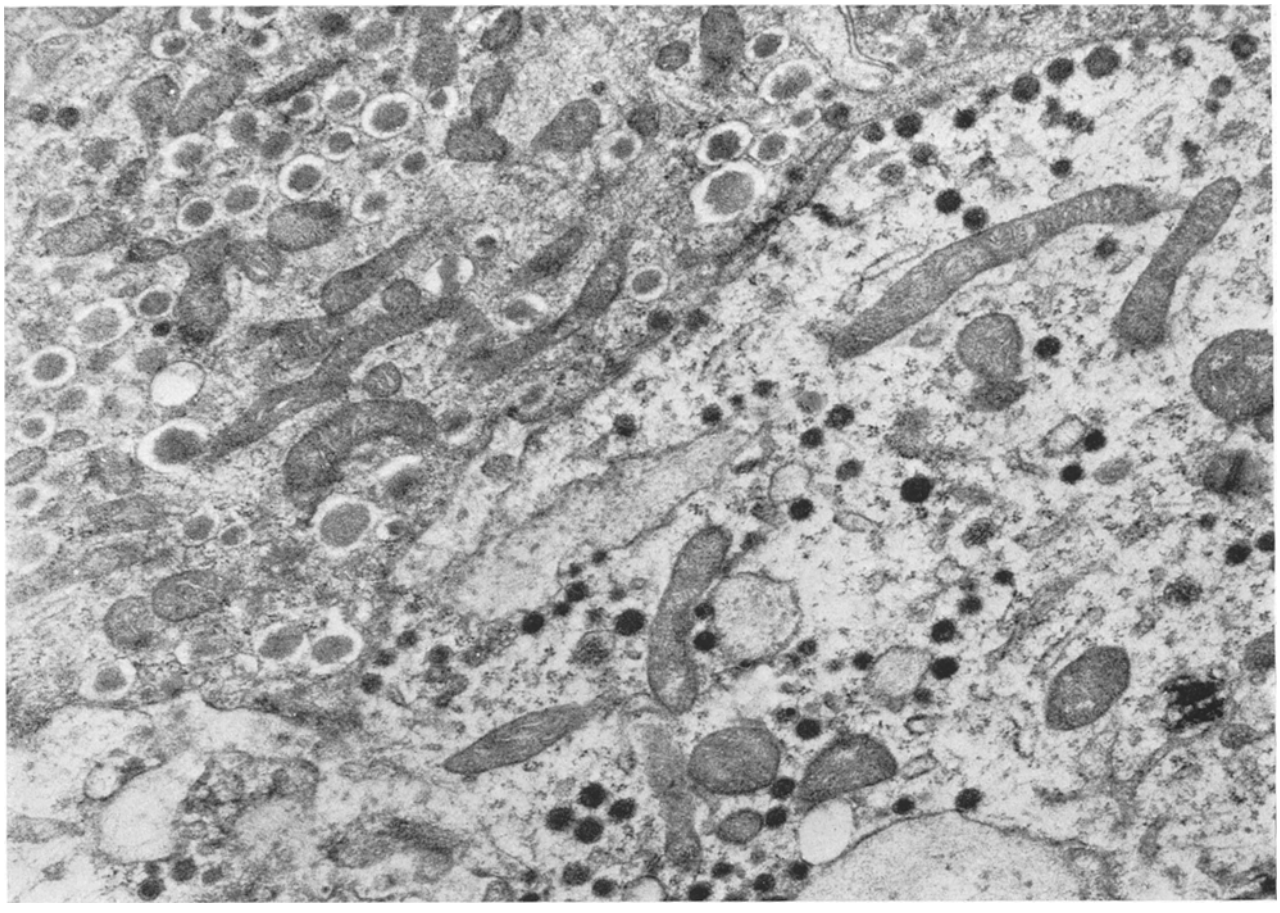

Fig. 7. Adjacent portions of two insulinoma cells, one showing typical beta-granules, the other atypical granules (Type II insulinoma). (Case No. 18, Table 1) ×24000

The ultrastructure of the islets of Langerhans of the normal pancreatic tissue revealed no abnormalities compared to the islets of non-diabetic persons without insulinoma. The frequency and type of beta-granules (multishaped or round dense granules and pale granules) varied from case to case. A- and D-cells showed their characteristic type of granules.

Discussion

The purpose of this study was to collect data from a large series of insulinomas regarding insulin content, PLC content, histology and ultrastructure. Previous studies reported upon only one or two of these parameters from a limited number of tumours. The results did not allow any conclusions regarding the frequency of abnormalities and the general functional defect of insulinomas responsible for the clinical picture of pernicious hyperinsulinism.

The present biochemical results confirm the observation [12, 15, 40, 52] that insulinoma cells contain less insulin than normal human B-cells. Great differences between single tumours were found (Fig. 2). The lowest values were found in the 2 carcinomas. Only one out of 30 insulinomas had an IRI concentration in the range of normal B-cells. Only 2 other cases have been reported with comparable high insulin levels [23, 29]. The decreased insulin contents cannot be attributed to the content of connective tissue or amyloid in most cases (Table 1). A low insulin content has also been found in microdissected cells of insulinoma tissue [15].

The biochemical result can be correlated with histological, immunohistological and ultrastructural findings. Only in the tumour with an insulin concentration comparable to islet B-cells (case No. 2) did practically all insulinoma cells react strongly with aldehyde-thionin, a specific stain for beta-granules. In the other tumours a variable number of cells were less intensively

stained or gave no reaction. The semi-quantitative evaluation of the histological findings correlated relatively well with the IRI concentration of the tumours (Table 1) with the exception of those tumours which contained, ultrastructurally, only atypical granules and were aldehyde-thionin negative (ultrastructural type III). However, in these cases the immunohistological reaction was positive and its intensity and distribution correlated well with the IRI concentration of the tumours. These findings indicate that nearly all insulin-stored in the tumour and in the pancreas exceed the mean insulin content of the normal pancreas.

The ultrastructural analysis of the insulinomas did not reveal any general abnormality which could explain this decreased storage capacity. Thirteen of 28 tumours had cells indistinguishable from normal islet B-cells, with the exception that a variable number of cells contained only few or no beta-granules, again consistent with the IRI concentration. This finding does not support the suggestion that the inability to

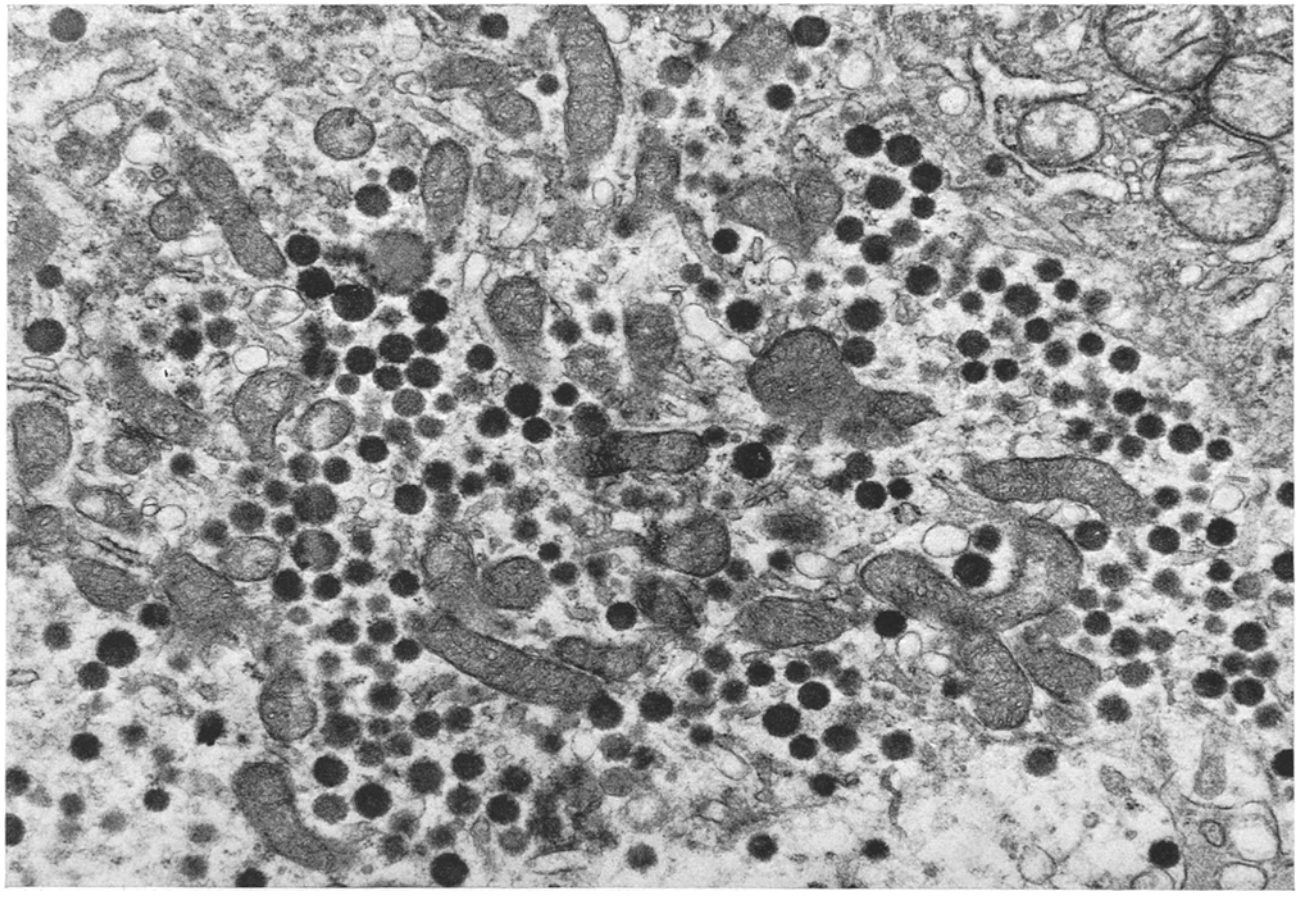

Fig. 8. Insulinoma cell with atypical granules, electrondense spherical granules usually smaller than beta-granules with or without a tightly fitting membrane (Type III insulinoma). There is no similarity to the granules of normal islet A-cells (having an electron dense central core) or the granules of D-cells (which are larger and less electron dense). (Case No. 16, Table 1) × 24000

omas contain cells which do not store insulin as competently as normal B-cells; this suggests a decreased storage capacity as a major characteristic of the tumour cell [12, 34]. The resulting uncontrolled release of insulin in the fasting state, i.e. in the presence of normal or even low blood glucose concentration and without additional physiological stimulation, could adequately explain the hypoglycaemic reactions of insulinoma patients. The total amount of extractable IRI from tumours rarely surpassed that found in the pancreas. Only in 11 cases did the sum of the insulin store insulin is reflected ultrastructurally by irregular and pleomorphic beta-granules [34]. Atypical beta-granules, in addition to normal beta-granules, were found in 7 cases (ultrastructural type II) and *only* atypical granules in 4 cases (ultrastructural type III). However, the mean tumour insulin concentration in these cases was only slightly lower, with considerable overlap, than the other groups (Table 2). Extremely low IRI concentrations were found in 4 virtually agranular tumours (ultrastructural type IV). From these data, it is concluded that the decreased storage

capacity of insulinomas is not uniform in all tumour cells. In some cases it is restricted to a few cells while in others (including the 2 carcinomas) to practically all cells. Thus, it may signify functional dedifferentiation. The agranular tumour cells revealed signs of high functional activity. Therefore, their identity with the agranular cells found during regeneration of islet tissue [4a] and in normal islets of lower vertebrates [10b], which are assumed to be precursors of the granulated islet cells [10c], must be regarded as an open question.

solely by ultrastructural findings. The same experience has been made with proven pancreatic gastrinomas, which frequently reveal secretory granules dissimilar to antral G-cells or islet D-cells [20, 53a, own unpublished observations]. In the present series, the diagnosis of an insulinoma was based upon biochemical analysis of the tumour extract (IRI concentration) in all cases and immunohistological investigation (1) in most cases (especially in those which were aldehyde-thionin negative).

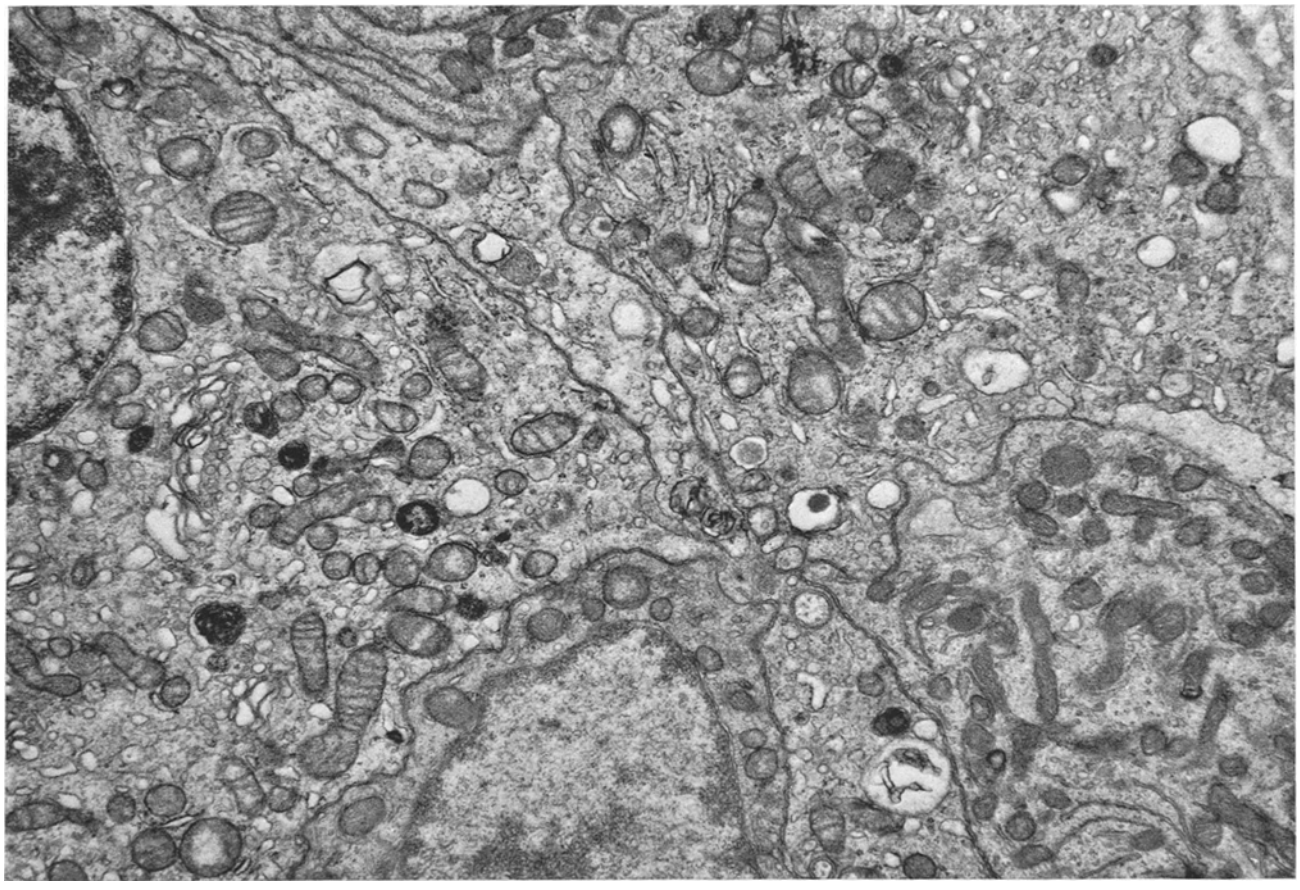

Fig. 9. Five adjacent portions of insulinoma cells containing only few typical beta-granules in the center. The remaining cells are agranular but show enhanced activity (prominent endoplasmic reticulum and Golgi apparatus, numerous cytoplasmic vesicles). (Case No. 29, Table 1) × 12000

The frequent finding of atypical granules in insulinoma cells and the occurrence of virtually agranular tumours shows that ultrastructural analysis of a pancreatic endocrine tumour alone is insufficient for definite identification. Eight of the 28 tumours studied ultrastructurally could not be identified as insulinomas by their ultrastructural appearance alone. Considering the occurrence of other hormone-producing tumours of the pancreas (gastrinoma, glucagonoma and diarrheogenic tumours) and of non-functioning endocrine tumours, caution is necessary in the identification of insulinomas

Regarding the genesis of the insulinomas, the present observations support the view that they originate from ductular elements [3,28]. In practically all cases close connections of the tumours to ductular structures were found. It cannot be decided, however, from these observations if the ductular structures consist of non-endocrine ductules or of immature endocrine cells, since regenerating and phylogenetically primitive and ontogenetically immature islet tissue has the tendency to form tubular structures [28b].

Extremely low insulin concentrations have been reported in the pancreas uninvolved by tumour in 2 of 3 [52] and 2 of 4 [12] cases. This has been explained by suppression of the normal islet tissue by the tumour induced hyperinsulinaemia, comparable to the effect of prolonged insulin injection in animals [4, 23, 27, 31, 32]. In the present series, the mean insulin concentration of the uninvolved pancreas of 21 patients was 2.0 ± 0.2 (range 0.49 — 4.85) U/g. These values did not differ from the insulin concentration in the pancreas of 3 non-diabetic subjects and are comparable with published data. The insulin concentration of the pancreas of non-diabetics has been reported being 1.7 (range 0.6 — 3.8) [48], 2.37 ± 0.14 (range 0.5 — 4.5) [54], and 2.15 ± 0.33 (range 1.0 — 3.9) [52] U/g pancreas. Thus, it can be concluded that the presence of an insulinoma usually does not affect the insulin concentration *i.e.* the storage of insulin in the normal islets. The biochemical data are supported by the histological, immunohistological and ultrastructural investigations of the islets of Langerhans, which revealed completely normal B-cells.

This fact is relevant to the evaluation of the percentage of proinsulin-like components (PLC) found in the pancreas and in the tumour since nothing yet is known about PLC percentages in the adult human pancreas. Considering the finding of normal insulin concentration in the pancreas of patients with insulinoma and the fact that the PLC percentage of 3 non-diabetics without insulinoma was in the range of the pancreatic glands of 9 patients with insulinoma suggests that all these PLC values are normal.

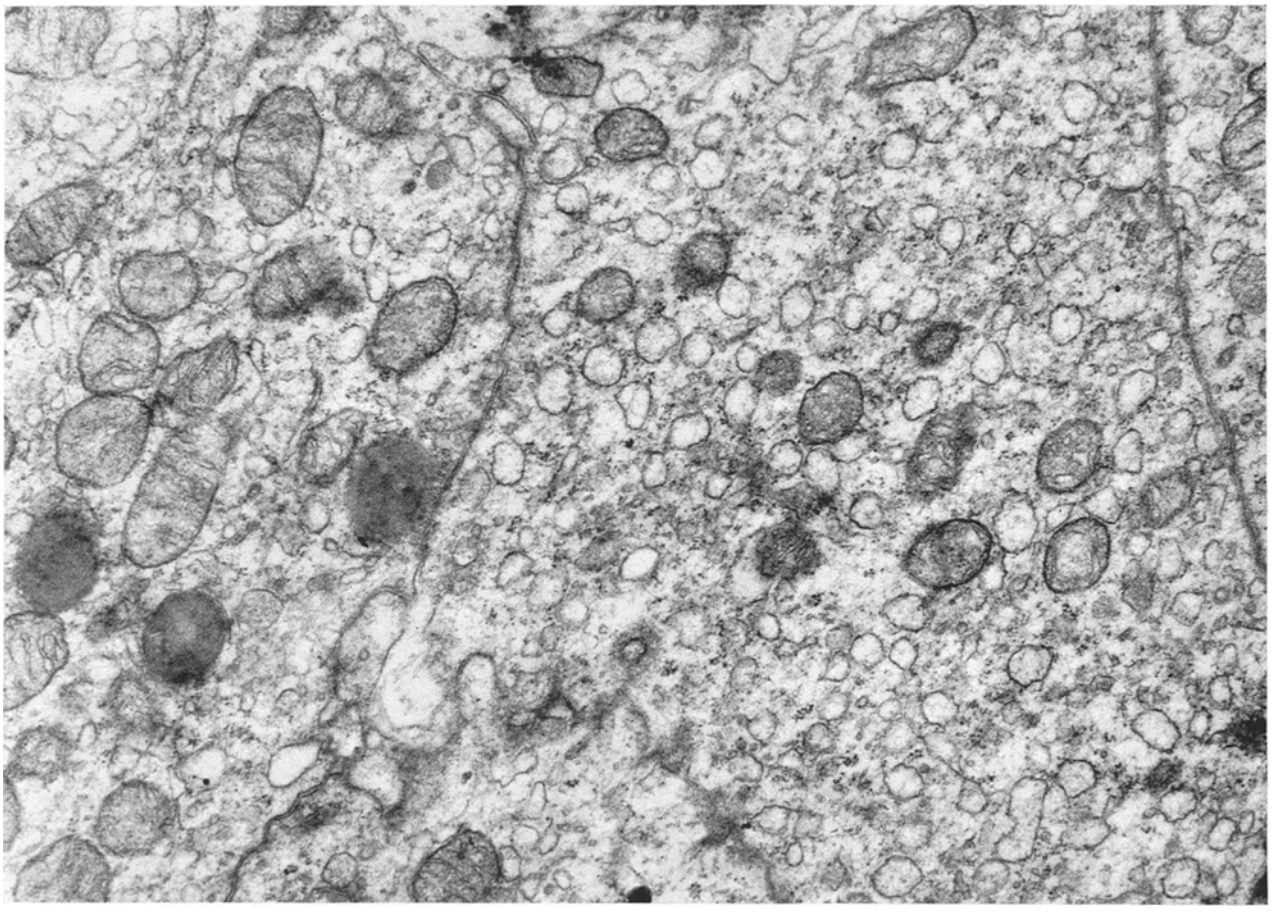

Fig. 10. Four adjacent portions of tumour cells of a virtually agranular insulinoma containing numerous small partially ribosome coated vesicles containing some electron lucent amorphous material (Type IV insulinoma). (Case No. 3, Table 1) × 24000

Very close to the findings in pancreas of rat and beef [50], pig and rabbit [30] and human fetuses [42], which contained less than 5% of the total extractable IRI as proinsulin, is the percentage of PLC in the adult human pancreas (mean 2.5 ± 0.2; range 1.7 — 4.8). The PLC percentage of the 19 investigated tumours were above these values ($m = 13.8 \pm 0.8$; range 5.3 — 22%). So far only 3 single cases of insulinoma have been analyzed for the PLC content of the tumour; the following values were found: 27% [36], 16% [18] and 5% [29]. In contrast, many studies have uniformly demonstrated that the percentage of PLC in the serum

is markedly elevated in cases of insulinoma [16, 17, 18, 22, 36, 49]. Twice, as exceptions, normal serum values have been reported [29, 46]. In the present study, the serum PLC were estimated in 6 cases and found to be markedly elevated (33 — 61%). It was much higher than in the tumour of the same cases (Table 1). This difference could be explained by the reported longer half-life of proinsulin than insulin in the plasma [45].

To explain the elevated serum PLC levels in insulinoma cases a relative or absolute deficiency of the converting enzymes [45] or a defective storage mechanism [19, 45] in the tumours have been suggested. No data have been presented to substantiate the first suggestion. The second explanation is supported by the fact that the percentage of serum PLC is higher in the fasting state than after stimulation (*i.e.* after release of stored insulin) and most markedly elevated in cases having poorly granulated tumour cells [19]. Since evidence is accumulating that the conversion of proinsulin to insulin takes place only in the Golgi apparatus and granules [24] a defect in storage capacity (*i.e.* granule formation) should lead to increased proinsulin levels. The finding of an increased percentage of PLC in all 19 investigated insulinomas and decreased storage of insulin in virtually all tumours strongly supports this hypothesis. By correlating the percentage of PLC in the tumours with the histological and ultrastructural findings, it is evident that the percentage of PLC is correlated to the number of poorly granulated or agranular tumour cells. The differences between the ultrastructural types I—IV are not significant (Table 2). Recent observations are in agreement with this concept. In contrast to an earlier report [47], it was shown that under stimulated conditions a preferential (non-granular) release of newly synthesized proinsulin/insulin occurs from isolated rat islets [53] and that incubated human insulinoma tissue has the capacity for a higher rate of proinsulin and insulin turnover than normal islet tissue [7]. Thus the increased PLC in the tumour and serum of patients with insulinoma seems to be the consequence of the decreased storage capacity of the tumour cells resulting in a shortened passage through the granular route or

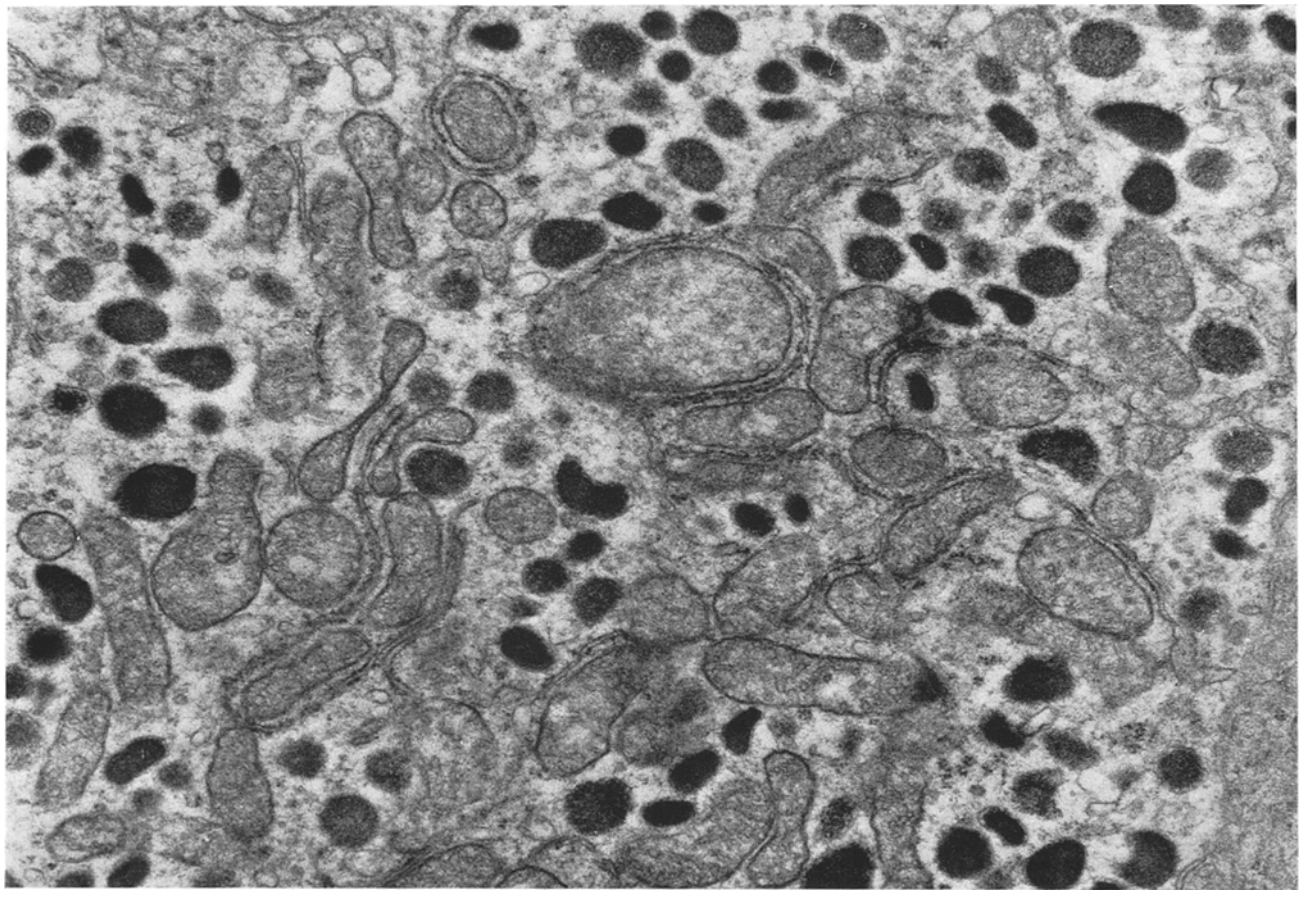

Fig. 11. EC-cell in an insulin-producing carcinoma of the pancreas. Comma-shaped granules of high electron-density. (Case No. 23, Table 1) × 24000

even a non-granular release of newly synthesized proinsulin and insulin.

The response to diazoxide was not directly correlated to the percentage of PLC. However, the 4 of the 24 tested patients who did not respond to this drug had the lowest insulin concentration in the tumour and revealed ultrastructurally only virtually agranular cells. Two were the carcinoma patients. Unresponsiveness to diazoxide has been described in patients with islet cell carcinoma [5, 18, 39]. Also a transplantable islet cell carcinoma of the golden hamster did not respond to diazoxide with a decrease of the plasma insulin levels [28a]. This tumour consisted primarily of agranular or sparsely granulated cells occasionally containing round granules of the ß-type [10a]. After incubation of human islet carcinoma tissue with diazoxide *in vitro* only proinsulin could be detected in the medium, suggesting insulin, but not proinsulin, release was inhibited by the drug [22a]. These findings support the hypothesis that diazoxide mainly blocks granular release from the B-cells [9, 10].

Acknowledgements. Only 17 patients of this series have been observed in the Department of Medicine, University of Göttingen. They were operated in the Department of General Surgery (Prof. H.J. Peiper). Three of these patients were diagnosed by Prof. K.F. Weinges (Department of Medicine, University of Homburg/Saar) and kindly transferred to us for further study.

Six cases were studied preoperatively by PD Dr. K. Bottermann (Department of Medicine, University of Munich) and operated by Prof. Zenker (Department of Surgery, University of Munich). One case was operated by Prof. E. Kern (Department of Surgery, University of Würzburg) and three by Prof. Berchtold (Department of Surgery, University of Bern). One patient with B-cell carcinoma previously published [37] has been operated by Prof. K. Kümmerle (Department of Surgery, University of Mainz). Three cases have been studied preoperatively by Prof. Fankhauser and Dr. J. Michl (Department of Medicine, University of Bern). One case was studied preoperatively by Prof. L. Kerp (Department of Medicine, University of Freiburg/Br.) and one by Prof. Gessler (IV. Department of Medicine, Municipal Hospital, Nuremberg). We wish to thank all colleagues for their generous support and co-operation.

We are most grateful to Miss H. Dörler, Mrs. B. Hillebrecht, Miss A. Nesslinger and Miss G. Schuman for their skilled technical assistance.

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