Studies on Islet Cell Regeneration, Hyperplasia and Intrainsular Cellular Interrelations in Long Lasting Streptozotocin Diabetes in Rats*

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Summary. Diabetes was induced in rats with various single i.v. doses of streptozotocin. The rats were killed 4–10 months thereafter. Marked hyperglycaemia, ketonaemia and a rise of plasma free fatty acids resulted. The weight increase was markedly reduced. There was a clear reciprocal correlation between the injected dose of the diabetogenic agent and plasma IRI. The same was true for the B-cell counts. There was a marked B-cell hypertrophy, whereas we found only slight indication for B-cell regeneration. In one animal a functioning B-cell adenoma developed and almost all the parameters tested were normalized. In the diabetic animals the A₂-cells were predominant in the islets of Langerhans and their mean nuclear diameters were increased. In the duodenal mucosa no increase in the number of glucagonocytes was found.

We interpret this activity of the glucagonocytes as a consequence of permanent and long-lasting hyperglycaemia.

Investigations sur la régénération et l'hyperplasie des cellules dans les îlots de Langerhans et sur les interrelations cellulaires intrainsulaires dans le diabète de longue durée provoqué par la streptozotocine chez le rat

Résumé. Par des doses différentes de Streptozotocine i.v. on provoque chez des rats un diabète d'une durée de 4 à 10 mois. On note une hyperglycémie sévère, une acétonémie et une augmentation modérée des acides gras. La prise de poids est diminuée de façon marquée. — Il existe une nette corrélation réciproque entre la quantité d'agent diabétogène injecté et l'insuline immunoréactive plasmatique (IRI). On trouve le même rapport entre le nombre des cellules B et la quantité de Streptozotocine. On note une corrélation directe entre le nombre des cellules B et l'IRI. — L'hypertrophie des cellules B est importante alors qu'on ne trouve que peu de signes de régénération des îlots et des cellules B. — Chez un des rats

on note l'apparition d'un adénome à cellules B fonctionnant, provoquant une normalisation du métabolisme glucidique. — Chez les animaux diabétiques, les cellules A₂ prédominent et le diamètre moyen des noyaux est augmenté. On ne note pas d'augmentation significative des glucagonocytes de la muqueuse duodénale.

Nous considérons que cette augmentation de l'activité des glucagonocytes est fonction de l'hyperglycémie permanente et de longue durée.

Untersuchungen zur Inselzell-Regeneration, Hyperplasie und zu intrainsulären zellulären Beziehungen während eines Langzeit-Streptozotocin-Diabetes bei Ratten

Zusammenfassung. Mit verschiedenen Dosen von Streptozotocin i.v. wird bei Ratten ein 4–10 Monate dauernder Diabetes induziert. Es resultiert eine schwere Dauerhyperglykämie, Acetonämie und ein mäßiger Anstieg der freien Fettsäuren. Die Gewichtszunahme ist wesentlich reduziert. Es besteht eine klare reziproke Korrelation zwischen injizierter Menge des diabetogenen Agens und Plasma-IRI. Ein gleicher Zusammenhang besteht zwischen der Zahl der B-Zellen und Streptozotocin. Eine direkte Korrelation findet sich für B-Zell-Zahlen und IRI. Die B-Zellen sind ausgeprägt hypertrophisch, während sich nur geringe Anhaltspunkte für B-Zellregeneration finden lassen. Bei einem Tier entwickelt sich ein funktionierendes B-Zelladenom mit konsekutiver Normalisierung des pathologischen Zuckerstoffwechsels. — Bei den diabetischen Tieren überwiegen die A₂-Zellen stark, und gleichzeitig ist der mittlere Kerndurchmesser vergrößert. In der Duodenalschleimhaut besteht keine signifikante Vermehrung der Glucagonocyten.

Wir interpretieren diese Aktivitätszunahme der Glucagonocyten als Ausdruck der fixierten und langdauernden Hyperglykämie.

Key-words: Streptozotocin-diabetes, B-cell hyperplasia, B-cell regeneration, A₂-cell hyperactivity, rat immunoreactive insulin, gut glucagonocytes, β -cytotrophy.

The still controversial reports on regenerative activity and neoformation of B-cells in diabetes [14, 20, 21, 22, 23, 28] and the discussions about the function of the glucagonocytes (A₂-cells) in hyperglycaemia have encouraged us to study these phenomena morphologically and biochemically in rats rendered chronically diabetic by streptozotocin. Streptozotocin is a

glucosamine derivative with well-known beta-cytotoxic properties [32, 17]. It has the advantage over alloxan that the toxic side effects are of relatively little importance and that a long survival is possible.

Material and Methods

Twelve Osborne-Mendel rats pure bred, male and 6–8 weeks old with an initial weight of 120–140 g survived for at least 4 months (mean 4.9 months) after a single intravenous injection of streptozotocin (kindly given to us by Dr. W. E. Dulin, the Upjohn Company, Kalamazoo, Mich. USA).

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The injected doses were 45–100 mg/kg body weight. Streptozotocin was dissolved in 0.9% saline and adjusted to pH 4 with citric acid as described by Junod *et al.* [17]. It was prepared immediately before use and injected intravenously.

The animals were fed with regular chow (Nafag pellets) and water *ad libitum*.

For practical purposes we formed two groups of diabetic rats. Group I comprised 8 animals injected with relatively low doses of streptozotocin i.v., (45–65 mg/kg); in Group II the rats received 85–100 mg/kg i.v. Group III consisted of 6 control animals of the same age. The animals were killed by decapitation in the fed state. Immunoreactive insulin (IRI) was determined in plasma by a double antibody method based on the Hales and Randle technique and using rat insulin standard (Novo Industries, Copenhagen). Plasma glucose was determined enzymatically, and acetone with the Galat reagent. Free fatty acids were assayed according to Gordon [8]. The pancreas was extirpated and 1 piece fixed in Bouin and another in Carnoy solution. The proximal duodenum was fixed in buffered 10% formalin. The fixed material was embedded in paraplast and 4–5 μ thick sections were stained.

types (see results section). In the duodenal mucosa, the number of Grimelius-positive cells is given as the number found in 100 completely and perpendicularly sectioned crypts as proposed by Funk *et al.* [6].

Karyometric studies were done in unselected islets using a magnification of 1250 times. 30 sectioned nuclei of each cell type were measured with a Zeiss ocular micrometer. By manipulation of the objective, the largest nuclear area was focused and the mean of two subsequent measurements determined. The largest and the smallest diameters were measured in oval nuclei. We corrected the diameters of non-spheric nuclei mathematically with the formula $\frac{a+b}{2}$, where a and b are the two arbitrary units. 7 units are equal to 10 μ .

Results

One single i.v. injection of streptozotocin resulted in a marked increase in glycaemia. Acetonaemia of varying degree was present in all animals and a slight rise of plasma free fatty acids (FFA) was observed (table 1). The weight increase of these growing animals was markedly retarded in the diabetic rats, although

Table 1

	Group I 45–65 mg/kg	Group II ^a 85–100 mg/kg	Group III controls	Unit
Glucose	659 $\pm$ 62	617 $\pm$ 38.5	165 $\pm$ 24.2	mg/100 ml
FFA	0.84 $\pm$ 0.1	0.71 $\pm$ 0.07	0.52 $\pm$ 0.13	μ eq/ml
IRI	32 $\pm$ 12.8	8.5 $\pm$ 3.5	141.2 $\pm$ 61.3	μ U/ml
terminal body weight	227.3 $\pm$ 35.4	156.5 $\pm$ 24.5	454 $\pm$ 36.7	g
B-cells	9.7 $\pm$ 4.33	3.1 $\pm$ 0.86	66 $\pm$ 3	% total islet cell count

^a The case with the B-cell adenoma is not included.

In the pancreas, the B-cells were demonstrated with the Gomori-Runge stain [25] or with Ivic's Victoria blue method [16]. A-cells may be recognized with this method as well, but to separate the two A-cell-types we used Hellman and Hellerström's method [13] for the identification of A₁-cells and the Grimelius method [9] for the A₂-cells (glucagonocytes). Serotonin cells were shown histochemically with the Masson-Hamperl technique [11] and with the Diazo coupling method [29]. Mast cells and Weichselbaum "Körnchen" [7] were identified with the Toluidine-blue method. The quantitative determinations of the cellular composition of the islets were performed in randomly selected islets by one of us not knowing the group to which the rats belonged. About 800 cells per pancreas were classified and differentially counted, the results for each cell type being given as a percentage of the whole cell population. Preliminary studies had shown that in our cases stereologic measurements were very difficult to interpret because of the existence of various B-cell

the food and water intake was excessive. In one animal (injected with 85 mg streptozotocin/kg), we found a normal terminal blood glucose value and the IRI was 78 U/ml. It weighed 365 g 10 months after the injection of the diabetogenic agent. Serial sections in the pancreas revealed the presence of a B-cell adenoma measuring 1.05 mm in diameter [Fig. 1]. We identified very few silver-positive cells (less than 1%) concentrated in one area of the adenoma. The largely overwhelming part of the tumour consisted of aldehyde-fuchsin positive cells. The remaining islets had the same repartition of islet cells (4.19% B-cells) as the other cases, injected with high doses of streptozotocin. In another case, most of the 85 mg streptozotocin/kg was injected paravenously. This animal did not differ from the controls either morphologically or biochemically. It is therefore not included in the statistical analysis.

There was a clear reciprocal correlation between the injected doses of the diabetogenic agent and plasma IRI (Fig. 2). The B-cell counts were related in the

same way to the amount of injected streptozotocin (Fig. 3a) and to the IRI in plasma (Fig. 3b). Most of the B-cells in strongly diabetic animals were very large, strongly degranulated and showed Weichselbaum "Körnchen", which are supposed to indicate an elevated synthetic activity of peptides [7]. Generally, a second B-cell population was observed, which appeared to be smaller in size and contained granules which were larger and more numerous (Fig. 4). In some

Fig. 1. B-cell adenoma developed in diabetic rat treated with 85 mg/kg streptozotocin i.v. For biochemical data see text. Gomori-Runge, 100:1

pancreases a few single cells were found scattered among the exocrine parenchyma which had the staining properties of B-cells (Fig. 5).

The percentage of B-cells in the islets was markedly reduced in all diabetic cases (Fig. 3) and their absolute number was also diminished since the total islet mass was not increased as may be derived from Fig. 6. Mitoses were not seen. Hydropic changes were infrequent (0–0.5% of total cell count). A few areas of fibrosis were found and mast cells were infrequent. The number of non-granulated cells which could not be classified according to type was between 1 and 7.5% (mean 4.1%) in the diabetic animals and between 0.6 and 2.5% (mean 1.5%) in the controls. The usual rat islet structure with peripheral arrangement of the A₂-cells and with the A₁-cells scattered in the region between the B and A₂-cells, appeared to be completely deranged in pancreases of diabetic rats.

The A₂-cells were predominant (Fig. 7) and scattered throughout the whole islet section surface. The mean nuclear diameters of the B-cells, measured in the Gomori-Runge stained sections, and of the A₂-cells (in Grimelius's stain) showed a marked increase in the

Fig. 2. Correlation between plasma IRI and injected doses of streptozotocin 5 months after treatment. Semilogarithmic scale. Open cycles show the SEM

Fig. 3 a. Correlation between the percentage of B-cells in the islets of Langerhans and the injected doses of streptozotocin 5 months after treatment. Semilogarithmic scale

b. Correlation between the percentage of B-cells and the plasma IRI. Logarithmic scale

diabetic animals (Fig. 8). We checked the validity of our results by measuring the A₁ and A₂-cell nuclear diameter together in the Gomori-Runge preparation. In the majority of cases the mean diameter of the A-cell

nuclei was a little larger in the slides with the Grimelius technique ($+4.3\%$; range -5 to $+14.7\%$), than stained in the Gomori-Runge preparations. For the

Fig. 4. Islet section from a diabetic rat after 45 mg/kg streptozotocin. 2 kinds of B-cells
1. large, almost completely degranulated ones
2. smaller, strongly granulated ones (arrow)
No. Ga 655, Gomori-Runge, 800:1

B-cells and slightly increased for A₂-cells 4.48 ± 0.40 units).

The duodenal argyrophil cells, as demonstrated by the Grimelius technique, were not significantly increased in the diabetic animals: 32.7 ± 19.7 cells/100 crypts in group I, 52.3 ± 19.5 in group II and 31.3 ± 14.7 in the control group III. Thin serial sections revealed that a part of the Grimelius-positive cells was also argentaffin; therefore more than one type of cells appears to be impregnated with Grimelius'silver reaction. The same is true for the Hellerström-Hellman stain.

Discussion

The diabetogenic effect of streptozotocin [32] is due to its marked β -cytotoxic activity [32, 17]. This action seems to be more specific than that of alloxan [17], and to have less general toxic side effects. Its action is also different from alloxan in several respects [35, 24]. Junod and coworkers [18] have recently shown in detail the effect of streptozotocin in rats immediately and until 28 days after injection of the agent. The most striking result in our study is the uniformity of all changes in diabetic animals as long as 5 months and more after the injection of streptozotocin. A dose response curve between streptozotocin and plasma IRI and B-cell counts in the pancreas was still present (Fig. 2, 3a). A steady state seems to be established between the number of B-cells and insulin secre-

Fig. 5. Isolated B-cell (arrow), scattered among exocrine parenchyma. No. Ga 152. 65 mg/kg streptozotocin. 6% islet B-cells. Glucose 773 mg/100 ml. IRI 27 μ U. Gomori-Runge, 800:1

cells in the control animals the difference was -5.7% (range -3.6 to -7.9%). In the islets of the case with the B-cell adenoma the mean nuclear diameter was the same as in the controls (4.52 ± 0.39 units) for

tion. Thus B-cell regeneration from insular or islet neoformation from extrainsular cells play no significant role, where as all criteria for a marked cellular hypertrophy are present. In one animal a B-cell

adenoma developed. This B-cell adenoma was not centred by tubular structures as in Boquist's case [2] of a short-time alloxan-diabetic chinese hamster. Mitoses appeared to be absent in the B-cells of our animals. Logothetopoulos [23] has, in fact, observed significant mitotic activity only in the early phase after streptozotocin injection (maximal in the first week). This activity was much less marked than in alloxan-treated rats. Therefore, it seems that B-cell neoformation and B-cell proliferation do not occur. The existence of a second B-cell type, already observed by Arison *et al.* [1], with stronger granulation and less marked hyperplasia cannot be as yet explained by our results. These authors interpreted their findings as signs of regeneration. The point in favour of a B-cell neoformation in our study is the finding of some aldehyde-fuchsin-positive cells among the exocrine pancreatic cells in some cases. Whether these single cells secrete insulin remains to be clarified.

It is well known that in certain animal species, such as mice, guinea pigs and rabbits, but also in infants of diabetic mothers [14, 20, 21, 22, 3, 5, 28] hyperglycaemia seems to be a stimulus for B-cell proliferation and neoformation. To our knowledge only Arison *et al.* [1] mention a slight regenerative activity in mild streptozotocin diabetic rats; Logothetopoulos *et al.* [23] found no indications for a sustained regeneration of B-cells in streptozotocin-diabetic mice. Lazarow [21], on the

Fig. 6. Number and diameter of islets in comparable areas of pancreas. Each vertical bar represents one animal, each dot represents one islet. Diameters are given in arbitrary units and are corrected to spheres. Group I: 45–65 mg/kg streptozotocin, Group II: 85–100 mg/kg, the bar to the right giving the case with the B-cell adenoma, the one to the left the case with partly paravenous injection. Group III: 2 control cases

Fig. 7. Islet of Langerhans in control animal (left) and in diabetic animal (right) after 45 mg/kg streptozotocin. Pre-dominance of A₂-cells in irregular clusters and few degenerated B-cells in the latter. No. Ga 640 and 655, Grimelius, 425: 1

other hand, even observed remission from long-term alloxan diabetes in rats, and already in 1954 Kinah and Haist [19] reported on marked B-cell hyperplasia

after chronic hyperglycaemia in this species. In dogs, islet neoformation and regeneration seems to be very unusual [22].

The metabolic abnormalities, i.e. hyperglycaemia, insulinopenia, polyuria and polyphagia are to be expected in the light of previous observations [17, 24]. The plasma FFA were not extremely elevated, a finding which is quite characteristic for long-lasting diabetes with a loss of body fat. A marked relative increase of A-cells was present in all the cases and these cells were mostly classified as A₂-cells. This has also been shown recently in guinea-pigs by Petersson *et al.* [30, 31]. This group also found in isolated islets that oxygen uptake after incubation with D-glucose was less marked in the streptozotocin-treated group, whereas succinate markedly stimulated the oxygen uptake by these A₂-cell rich islets. The average diameters of the A₂- and the B-cell-nuclei were significantly increased (Fig. 8a and b) which suggests an increased protein-synthetic activity [15, 28, 26, 34].

Fig. 8a. Correlation of A₂- and B-cell nuclear diameters in streptozotocin-treated rats

Orci *et al.* [27] reported an increased number of lysosomal bodies which contain the specific α_2 -granules in A₂-cells of streptozotocin-treated diabetic rats. The authors favour the hypothesis that this reflects destruction of unneeded glucagon. However, they discuss the possibility that this increase of lysosomes may have to do with solubilization of stored hormone prior to secretion or with a process favouring "cellular dedifferentiation returning the cell to a potentially more versatile state". Our results as well as those of Petersson's group [30, 31] seem to support the latter possibilities, because both A₂ and B-cells seem to be quite active. In a normal individual, during a glucose tolerance test, pancreatic glucagon falls and gut glucagon in plasma increases almost simultaneously with the rise of the IRI [12]. Under this condition, gut glucagon does not seem to mobilize glucose but rather insulin. The same conclusion was drawn from the faster insulin release during oral than i.v. glucose tolerance test. Possibly other agents, such as secretin, may also play a role. Samols *et al.* [33], on the other hand, postulated that gut glucagon is primarily hyperglycaemic and pancreatic glucagon primarily insulinogenic and hyperglycaemic only in the second place; *in vitro* the insulin

releasing action of pancreatic glucagon was demonstrated in rat pancreas even in the absence of glucose [4]. Under pathologic conditions, as in the present study, characterized by a strong insulinopenic hyperglycaemia, the pancreatic glucagonocytes seem to be very active and might be mainly β -cytotrophic. On the other hand, the duodenal glucagonocytes are neither increased nor activated. However, our quantitative results of the duodenal cell analysis are rather difficult to interpret, because at least one more cell type, the serotonin-cell, was also stained with the present method. The glycogen-mobilizing activity of glucagonocytes might be of importance even in these cases. In

b. Mean diameters of A₂-cell nuclei

Group I: 45–65 mg/kg streptozotocin i.v.,
Group II: 85–100 mg/kg streptozotocin i.v.,
Group III: controls

7 units = 10 μ

$$\delta = \frac{s}{n}$$

t_1 versus 3 < 0.05 t_2 versus 3 < 0.005

conclusion, there is rather good evidence for a low regenerative and neoformative activity of pancreatic B-cells in streptozotocin-diabetic rats. The remaining B-cells appear very active, possibly as a result of stimulation by the pancreatic glucagonocytes and/or high extracellular glucose concentration. The development of a functioning B-cell adenoma in one of the animals may be a consequence of chronic stimulation of a tissue which, otherwise, has lost its regenerative activity. On the other hand, we have no indications for an exhaustion of the B-cells.

Further studies are needed to answer the question whether the stimulation of the remaining B-cells is mediated through the A₂-cells or the sustained hyperglycaemia in the streptozotocin-treated animals.

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Literature

1. Arison, R.N., Ciaccio, E.I., Glitzer, M.S., Cassaro, J. A., Pruss, M.P.: Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. *Diabetes* **16**, 51–56 (1967).
2. Boquist, L.: Alloxan Administration in the Chinese Hamster. *Virchows Arch. Abt. B. Zellpath.* **1**, 157–168 (1968).
3. Cavallero, C.: Application de la méthode colchicinique à l'étude du diabète alloxanique chez le rat. *Rev. belge Path. Méd. Expér.* **18**, 323–329 (1947).
4. Derrim, S., Recant, L.: Effect of glucagon on insulin release in vitro. *Lancet* **1966 II**, 1227–1228.
5. Friedman, N.B., Marble, A.: Island hyperplasia in partially depancreatized rats. *Endocrinology* **29**, 577–583 (1941).
6. Funk, H.U., Weber, E., Hedinger, Chr., Hardmeier, Th.: Die Zahl der argentaffinen Zellen des menschlichen Verdauungstraktes unter normalen und pathologischen Bedingungen. *Virchows Arch. Path. Anat.* **340**, 289–303 (1966).
7. Gepts, W.: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* **14**, 619–633 (1965).
8. Gordon, R.F., Cherkas, A., Gates, N.: Unesterified fatty acid in Human Blood Plasma. II. The transport function of unesterified fatty acid. *J. clin. Invest* **36**, 810–815 (1957).
9. Grimelius, L.: A silver nitrate stain for A₂ cells in human pancreatic islets. *Acta Soc. Med. upsalien.* **73**, 243–276 (1968).
10. Hales, C.N., Randle, P.J.: Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* **88**, 137–146 (1963). *Lancet* **1963 I**, 200.
11. Hamperl, H.: Über die „gelben (chromaffinen)“ Zellen im gesunden und kranken Magendarmschlauch. *Virchows Arch. path. Anat.* **266**, 509–548 (1927).
12. Hedinger, L.: Determination of pancreatic and gut glucagon in plasma. Abstr. 5th annual meeting of the Europ. Assoc. for the study of Diabetes, Montpellier, 1969.
13. Hellerström, C., Hellman, B.: Some aspects of silver impregnation of the islets of Langerhans in the rat. *Acta endocr. (Kbh.)* **35**, 518–532 (1960).
14. Hellman, B., Hellerström, C.: Histology and histophysiology of the islets of Langerhans in man. In: *Diabetes mellitus*, Vol. I, ed. E.F. Pfeiffer. München: Lehmann's Verlag 1968.
15. Hultquist, G.T.: Nuclear size in the cells of Langerhans' islets during starvation in the rat. *Acta anat.* **49**, 281–287 (1962).
16. Ivic, M.: Neue selektive Färbungsmethode der A- und B-Zellen der Langerhans'schen Inseln. *Anat. Anz.* **107**, 347–350 (1959).
17. Junod, A., Lambert, A.E., Orci, L., Pictet, R., Gonet, A.E., Renold, A.E.: Studies on the diabetogenic action of streptozotocin. *Proc. Soc. exp. Biol. Med.* **126**, 201–205 (1967).
18. Junod, A., Lambert, A.E., Stauffacher, W., Renold, A.E.: Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. *J. clin. Invest.* **48**, 2129–2139 (1969).
19. Kinash, B., Haist, R.E.: Continuous intravenous infusion in the rat, and the effect on the islets of Langerhans of the continuous infusion of glucose. *Canad. J. Biochem. Physiol.* **32**, 428–433 (1954).
20. Lazarow, A.: Spontaneous recovery from alloxan diabetes in the rat. *Diabetes* **1**, 363–371 (1952).
21. Lazarus, S.S., Volk, B.W.: The effect of protracted glucagon administration on blood glucose and on pancreatic morphology. *Endocrinology* **63**, 359–371 (1958).
22. — Volk, B.W.: The pancreas in human and experimental diabetes. N.Y. London: Grune and Stratton 1952.
23. Logothetopoulos, J., Rorovsky, S.: Mitotic activity of islet cells in alloxan and streptozotocin diabetic mice studied by autoradiography. *Diabetes* **17**, Suppl. **1**, 306 (1968) Abstr.
24. Mansford, K.R.L., Opie, L.: Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by alloxan. *Lancet* **1968 I**, 670–671.
25. Mueller, I., Runge, W., Ferner, H.: Cytologie und Gefäßverhältnisse des Inselorgans bei der Ente. *Z. mikr.-anat. Forsch.* **62**, 165–186 (1956).
26. Oehlert, W., Schultze, B.: Die Kerngröße als Ausdruck der synthetischen Aktivität des Kerns. *Beitr. path. Anat.* **123**, 101–113 (1960).
27. Orci, L., Junod, A., Renold, A.E., Rouiller, C.: Granulolysis in A-cells of endocrine pancreas in spontaneous and experimental diabetes in animals. *J. cell Biol.* **38**, 462–466 (1968).
28. Patent, G.J., Alfert, M.: Histological changes in the pancreatic islets of Alloxan treated mice, with comments on β -cell regeneration. *Acta anat.* **66**, 504–519 (1967).
29. Pearse, A.G.E.: In: *Histochemistry*. London: A. Churchill 1961.
30. Petersson, B., Hellerström, C., Gunnarsson, R.: Metabolic studies of pancreatic A-cells. Abstr. annual meeting Europ. Assoc. for the study of Diab. Montpellier. 1969.
31. — — — Structure and metabolism of the pancreatic islets in streptozotocin treated guinea-pigs. *Hormone. Met. Res.* **2**, (1970), in press.
32. Rakieten, N., Rakieten, M.L., Nadkarni, M.V.: Studies on the diabetogenic action of streptozotocin (NSC-37917). *Cancer Chemoth. Rep.* **29**, 91–98 (1963).
33. Samols, E., Tyler, J., Megyesi, C., Marks, V.: Immunochemical glucagon in human pancreas, gut and plasma. *Lancet* **1966 II**, 727–729.
34. Steiner, H., Hedinger, Chr.: Storage of acidophilic granules in the pituitary in experimental hyperglycaemia. *Diabetologia* **6**, 373–378 (1970).
35. Veleminsky, J., Burr, I., Gutzeit, A., Beaven, D., Stauffacher, W.: Streptozotocin-diabetes prevention by nicotinamide and comparisons with alloxan diabetes. Abstr. annual meeting Europ. Assoc. for the study of Diab. Montpellier. 1969.

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