Virchows Arch. A Path. Anat. and Histol. 371, 227—241 (1976)

Morphologic Effects of Diazoxide and Diphenylhydantoin on Insulin Secretion and Biosynthesis in B Cells of Mice*

G. Bommer, H.-J. Schäfer, and G. Klöppel

Institute of Pathology, University of Hamburg, Germany

Received April 20, 1976

Summary. The action of diazoxide, an antidiuretic agent, and diphenylhydantoin, an antiepileptic (DPH), both with strong hyperglycemic side effects on the pancreatic B cells, was examined by electron microscopy and cytochemistry, with the following findings.

1. Effects on secretory apparatus: the severe hyperglycemic syndrome following a single injection of diazoxide (200 mg/kg) or DPH (150 mg/kg) did not change the granularity of the B cells. Ultrastructurally a marked increase of lysosomal digestion of secretory granules (crinophagy) was observed in almost all B cells. Crinophagy may be regarded as a result of an impaired discharge of secretory granules during simultaneous maintenance of biosynthesis. It is also possible that changes of the electrophysical properties of the granule surfaces may play an additional role in crinophagy.

2. Effect on synthesizing apparatus: in B cells subtotally degranulated by the injection of anti-insulin serum (AIS), regranulation occurred more rapidly after the additional administration of diazoxide or DPH than without these compounds. This fact may imply that, under the hyperglycemic conditions tested, diazoxide or DPH have no effect on the synthesizing capacity of the B cells.

Key words: Inhibition of insulin secretion — Diazoxide — Diphenylhydantoin — B cell morphology — Crinophagy.

Introduction

Diazoxide, a benzothiadiazine derivative, and diphenylhydantoin (DPH), a hydantoin derivative, show strong hyperglycemic effects (Wolff et al., 1963; Fariss and Lutcher, 1971). Both substances cause direct inhibition of insulin secretion (Levine et al., 1972). A marked crinophagy in B cells was found to be the morphologic equivalent of the acute, diazoxide-induced inhibition of insulin secretion (Creutzfeldt et al., 1969). After a protracted application of diazoxide, degranulated inactive B cells were found. Similar morphologic investigations of DPH-treated B cells have not yet been made.

The present study deals with the short-term effect of diazoxide on the process of insulin secretion in B cells of mice, and compares these findings with the B cell changes following a similar DPH treatment. Moreover, in order to discern a possible inhibitory effect on insulin biosynthesis by morphologic methods, we conducted a regranulation experiment. For this purpose the B cells of animals were at first stimulated and subtotally degranulated by application of anti-insulin serum (AIS). Subsequently, release of insulin was inhibited by means of diazoxide or DPH and the regranulation was traced. The degree and velocity of the regranulation were regarded as indicators of the biosynthetic activity of B cells under diazoxide and DPH treatment.

Materials and Methods

The investigations were conducted on male albino mice weighing 25–30 g. The animals were kept under standard conditions with an Altromin® standard diet and free access to water. All experiments were performed in nonfasting animals.

* Supported by Deutsche Forschungsgemeinschaft, SFB 34, Endocrinology, Hamburg

Table 1. Grouping and treatment

Groups	n	Treatment			Duration
1	9	Diazoxide			90, 180, 360 min
2	9	DPH			90, 180, 360 min
3	9	0.9% NaCl			90, 180, 360 min
4	20	AIS	AIS		6, 9, 12, 18, 24 h
5	20	AIS	AIS	Diazoxide	6, 9, 12, 18, 24 h
6	20	AIS	AIS	DPH	6, 9, 12, 18, 24 h
Injection		▲0 h	▲3 h	▲6 h	

Substances. Diazoxide (Hypertonalum®; Byk-Essex, München) was injected i.p. in single doses of 200 mg/kg body weight. Diphenylhydantoin (Diphenhydan, Desitin, Hamburg) was administered in single doses of 140 mg/kg i.p. Anti-insulin serum (AIS) from guinea pigs against bovine insulin was injected i.p. in single doses of 0.5 ml.

Grouping. The following 6 groups were formed (Table 1):

Groups 1–3: the animals received single injections of diazoxide (group 1) and DPH (group 2) in doses as mentioned above. Three animals were sacrificed after 90, 180 and 360 min. The controls (group 3) received 0.5 ml 0.9% NaCl.

Groups 4–6: each animal was injected twice with anti-insulin serum (AIS) in the doses given above at the beginning of the experiment and 3 h after. While group 3 remained as a control group, groups 4 and 5 received diazoxide or DPH in addition to AIS 6 h after the beginning of the experiment. Four animals of each group were killed after 6, 9, 12, 18, and 24 h.

Determination of Plasma Glucose Levels. Prior to the beginning of the experiments and at sacrifice blood samples of each animal were taken from the retroorbital venous plexus. In an additional study blood samples were drawn from mice ($n = 15$) 30, 90, 180, 360 min, and 12 h after injection of diazoxide or DPH (Fig. 1). Plasma glucose was determined using the glucose oxidase method (Boehringer, Mannheim).

Determination of Plasma Immunoreactive Insulin. Values of immunoreactive insulin (IRI) were determined in the animals of group 1 and in some of group 3 by the method of Zaharko and Beck (1968) using rat insulin as the standard.

Light microscopy. After decapitation of the animals under ether anesthesia a piece of pancreatic tissue was fixed in Bouin's solution. From paraffin-embedded material 7 μ-thick sections were stained with hematoxylin eosin, periodic acid Schiff, and Gomori's aldehyde fuchsin (AF). B-cell secretory granulation was estimated semiquantitatively through evaluation of the AF-staining intensity according to the following scale: 1 = weak, 2 = moderate, 3 = strong.

Electron Microscopy. Immediately after removal pancreatic tissue was cut into small pieces and fixed in 2.1% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4). After rinsing in cacodylate buffer the tissue was postfixed in 1% buffered osmic acid, again repeatedly rinsed in the cacodylate buffer, then dehydrated in ethanol and propylenoxide, and finally embedded in Epon 812. Ultrathin sections were cut with a Reichert ultramicrotome OM U2, stained with uranylacetate and lead citrate, and studied with Zeiss EM 9.

For ultracytochemical demonstration of acid phosphatase in B cells pancreatic tissue was fixed for 2 h in glutaraldehyde. Subsequently the tissue was enclosed in agar, and 40 μ sections were prepared with a Sorvall tissue chopper. Under the stereomicroscope those sections which contained islets, were singled out and examined further. These sections were then incubated for 20 min at 37°C in a modified solution (Ericsson and Trump, 1965), which consisted of 60 mg sodium α-glycerophosphate (Serva, Heidelberg) in 17 ml bidistilled water

and 5 ml stock solution. The stock solution consisted of 2.85 g dehydrated sodium acetate 0.7 g lead nitrate (cryst.), 0.5 ml glacial acetic acid, and 56 g sucrose. The solution was filled up to 150 ml with bidistilled water. After incubation and rinsing three times in 7.5% sucrose, the tissue was dehydrated as mentioned above, embedded in Epon 812, cut with an ultramicrotome, and stained with lead citrate.

Results

Effects of Diazoxide (Group 1)

The single injection of diazoxide resulted in a temporary hyperglycemic syndrome, which persisted over a 12-h period (Fig. 1). A peak value of 614 mg% was measured after 360 min, while at the same time the serum insulin levels decreased to almost immeasurable values.

The B cells demonstrated a marked to strong granulation throughout the entire investigation. When ultrastructurally viewed the secretory granules of the B cells were uniformly distributed over the cytoplasm. Frequently to be observed were barlike, in part arcuate deposits of uniform electron-opaque material in the halos of single secretory granules (Fig. 2). Moreover, numerous lysosomes were found containing secretory granules. These lytic bodies were classed as multigranulated bodies and dense bodies. The multigranulated bodies consisted of several densely aggregated but nevertheless distinctly separable granule cores, which were sur-

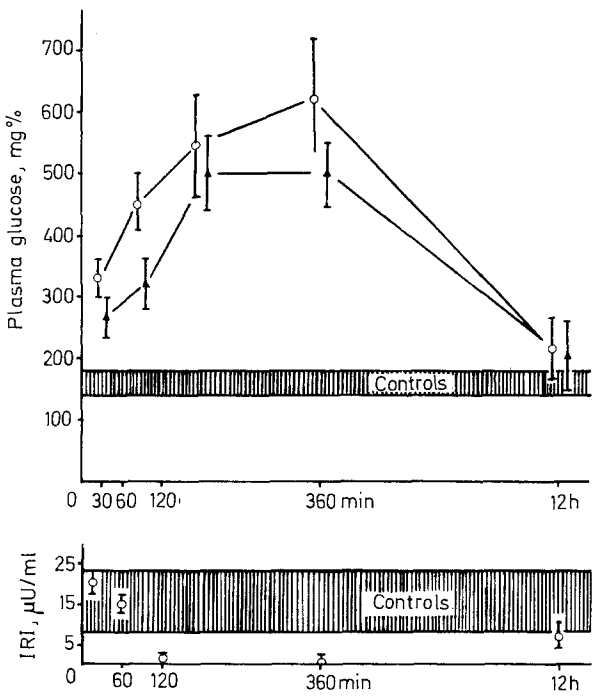

Fig. 1. Mean values (± SDM) for plasma glucose in mice after a single injection of diazoxide (○) or DPH (▲). Plasma immunoreactive insulin levels (± SDM) in mice treated with diazoxide

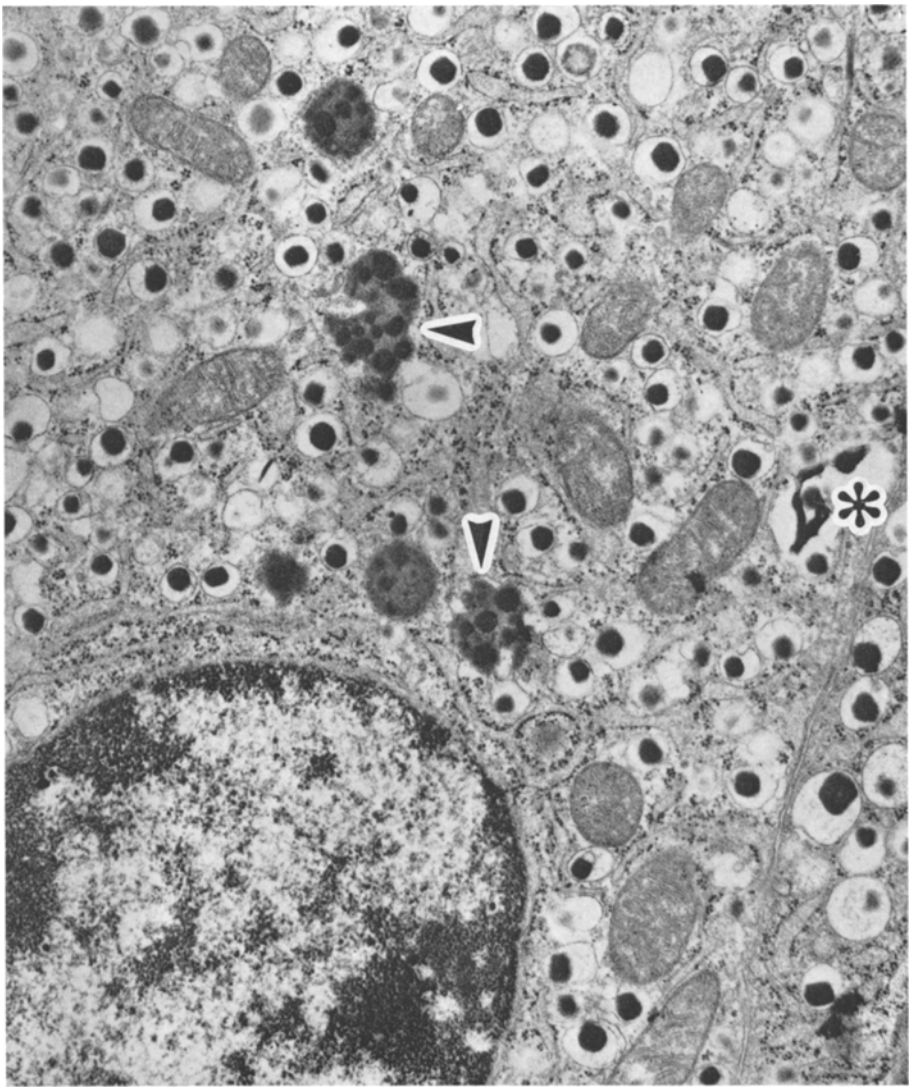

Fig. 2. B cell of mouse 90 min after injection of DPH. Well-granulated B cell with multigranulated bodies (arrow). Single barlike deposits of electron dense material in the halos of secretory granules (asterix). ×16,400

rounded by a single limiting membrane. The dense bodies were round electron-dense figures containing blurred granule residues within a dense matrix. Transitions were present between multigranulated and dense bodies. Furthermore, aggregates of secretory granules with fused membranes were found, which probably represented precursors of multigranulated bodies. Fusions of aggregated secretory granules with other organelles, e.g., lysosomes could not be observed. On the other

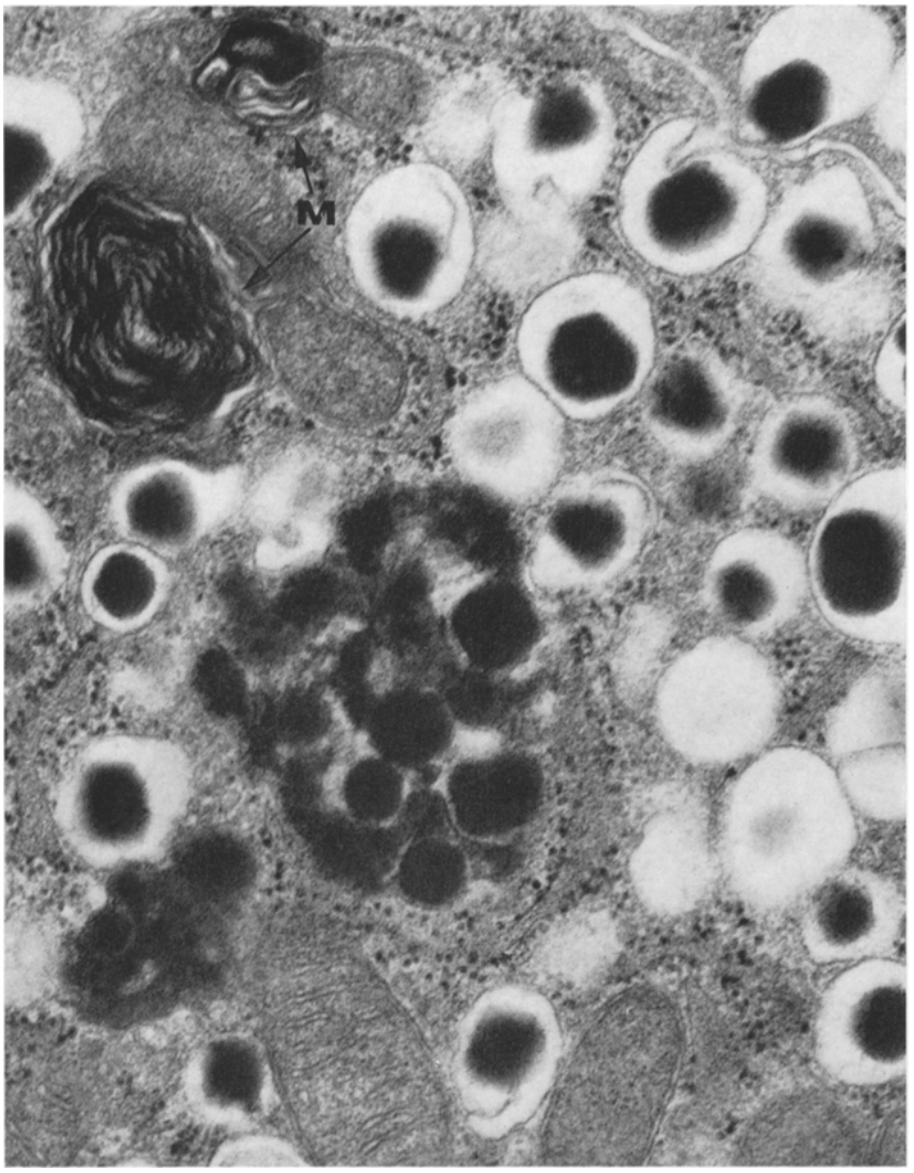

Fig. 3. Part of a B cell 360 min after diazoxide injection. Between secretory granules multigranulated bodies are present with blurred surrounding membranes and beginning disintegration of the granule cores. Adjacent are myelin figures (*M*). ×57,000

hand, incorporation of single secretory granules into dense bodies was occasionally seen. Granule aggregates, multigranulated and dense bodies occurred in animals sacrificed at all times. Some of the multigranulated and dense bodies showed signs of degradation from dissolution of their electron-dense content and their limiting

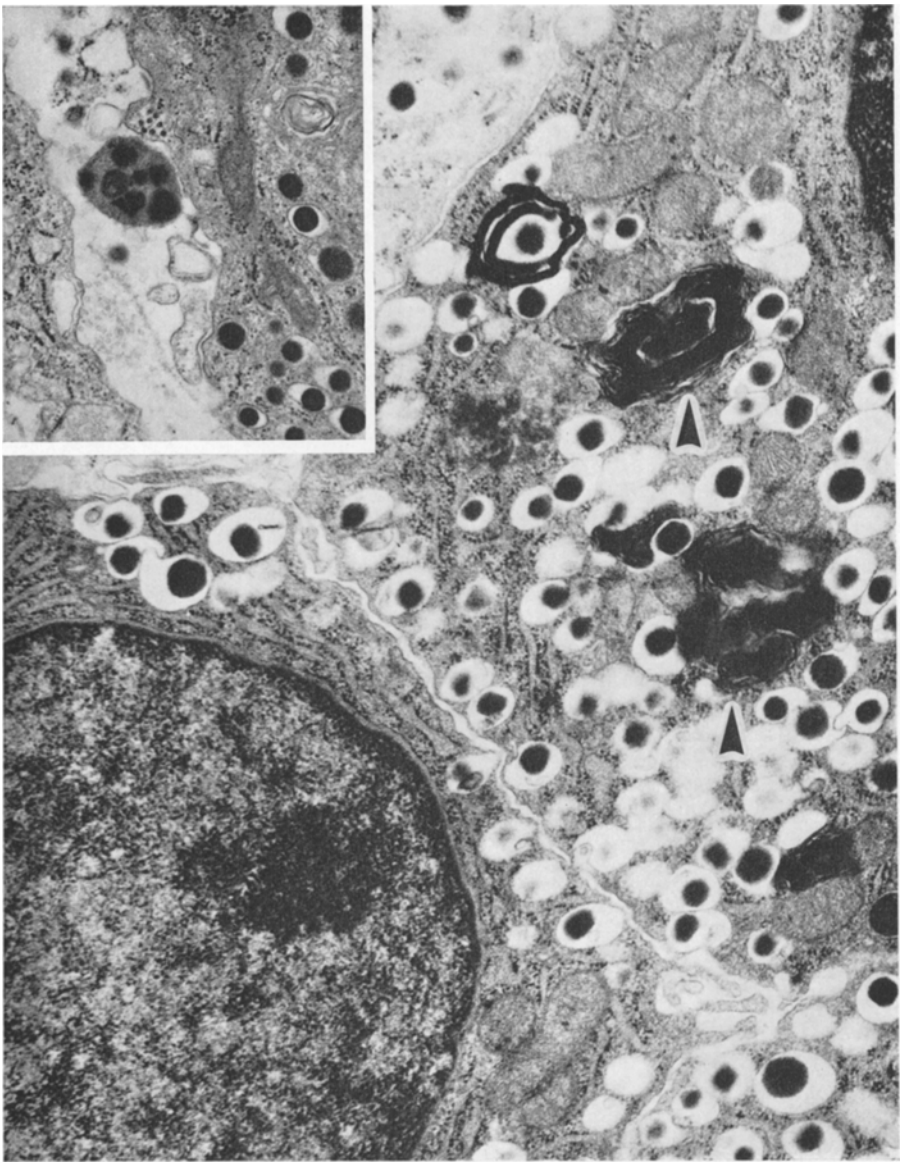

Fig. 4. B cells 360 min after diazoxide injection: in cytoplasm are disintegrating multigranulated bodies, some of which are in process of changing to myelin figures (arrow). Inlet: Multigranulated body in extracellular space. ×19,500

membranes (Fig. 3). This was most obvious at 360 min. Finally, they appeared to be transformed in myelin figures (Fig. 4). Single multigranulated bodies were observed in the extracellular space, suggesting an occasional exocytosis of lytic bodies. The Golgi complexes as well as the RER remained unchanged.

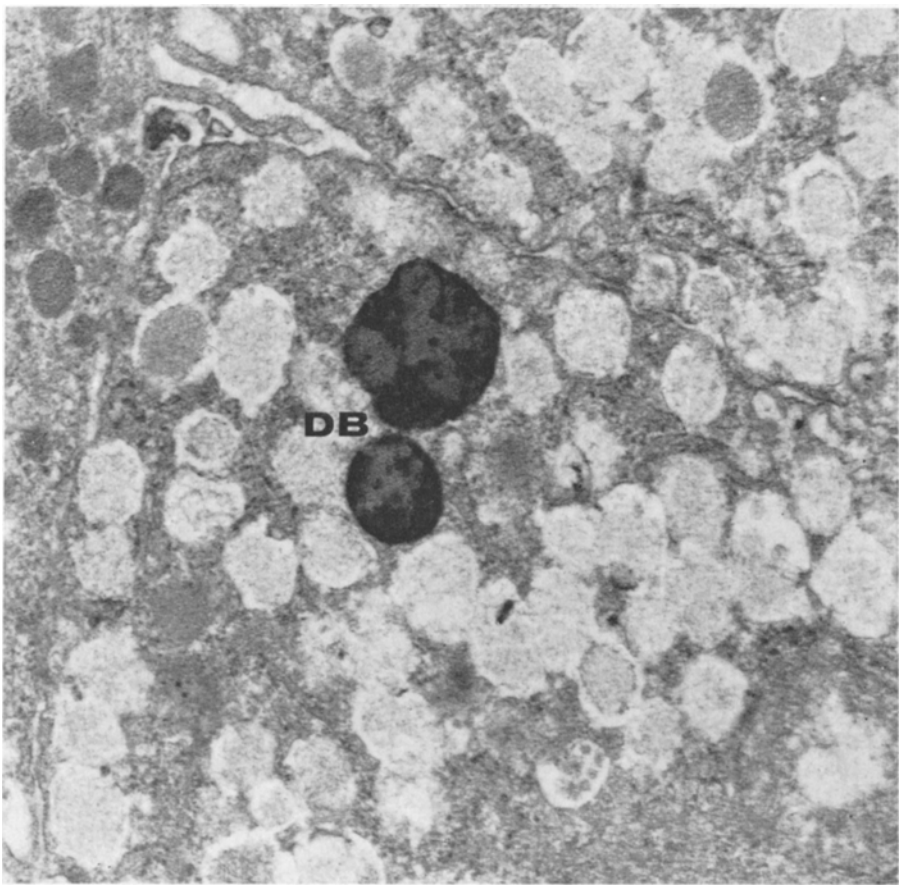

Fig. 5. B cell 90 min after diazoxide injection: strong acid phosphatase activity within dense body (*DB*) containing secretory granulelike material. ×26,000

Effects of Diphenylhydantoin (Group 2)

As after diazoxide, DPH application leads to a rapid and reversible increase in plasma glucose, reaching a maximum of about 500 mg% after 180 min (Fig. 1). Original values were restored after 12 h.

Histologically, the B cells remained heavily granulated throughout the hyperglycemic syndrome. When viewed ultrastructurally the B cells were found to be well granulated. Analogous to diaxoxide treatment crinophagy phenomena with multigranulated and dense bodies were also found. All together, however, these processes seemed less frequent and less marked when compared with diazoxide-treated animals.

Ultracytochemical evidence of acid phosphatase activity was always markedly positive in the multigranulated and dense bodies of B cells in diazoxide or DPH-treated animals (Fig. 5). A weak reaction was also found in the Golgi complexes, whereas the granule halos remained unstained.

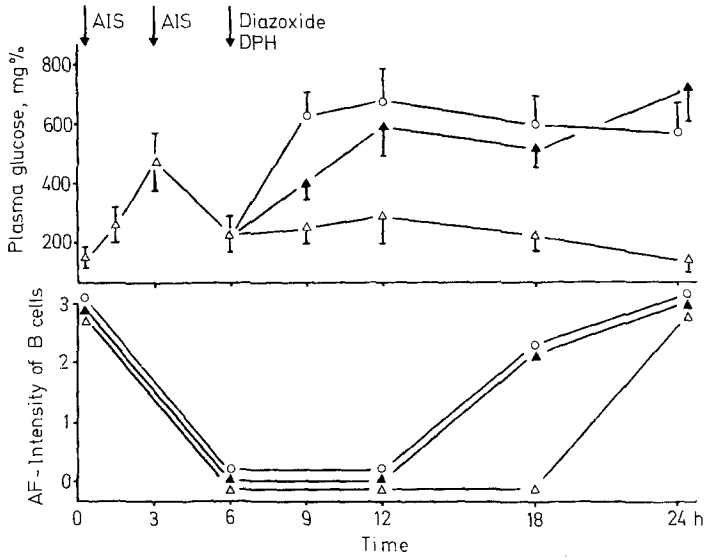

Fig. 6. Mean values for plasma glucose (± SDM) and B-cell granulation in mice during treatment by AIS (△) applied twice and subsequent diazoxide (○) and DHP (▲) injections respectively. Degree of B-cell granulation is evaluated by intensity of aldehyde fuchsin (*AF*) staining (1 = weak, 2 = moderate, 3 = strong)

Controls (Group 3)

The B cells of the normoglycemic controls were well granulated. At the ultrastructural level, signs of lysosomal digestion of secretory granules could only be identified singly.

Effects of Anti-Insulin Serum and Diazoxide or DPH (Group 4–6)

To estimate better biosynthesis of insulin after diazoxide and DPH application, B cells, which were subtotally degranulated by AIS injection before treated with diazoxide or DPH, were examined as to their ability to regranulate under these conditions.

Repeated injections of AIS (group 4) brought about a modest hyperglycemia with average values of about 250 mg% (Fig. 5). These values remained almost constant over a period of 18 h and returned to original values after 24 h. In spite of this moderate hyperglycemia a marked B cell degranulation, lasting about 12 h, was observable on histologic examination (Figs. 6 and 7). At the ultrastructural level, in addition to the degranulation, a marked activation of the biosynthetic apparatus appeared, showing an enlargement of the Golgi complexes and a RER hyperplasia.

AIS and subsequent diazoxide or DPH injection (groups 5 and 6) induced a marked hyperglycemia with average values of about 600 mg%, which persisted until the end of the experiment (Fig. 6). Histologically, the initial subtotal degranulation resulting from AIS treatment changed into a rapidly increasing regranulation 9 h after diazoxide or DPH injection. Thus the granularity of the B

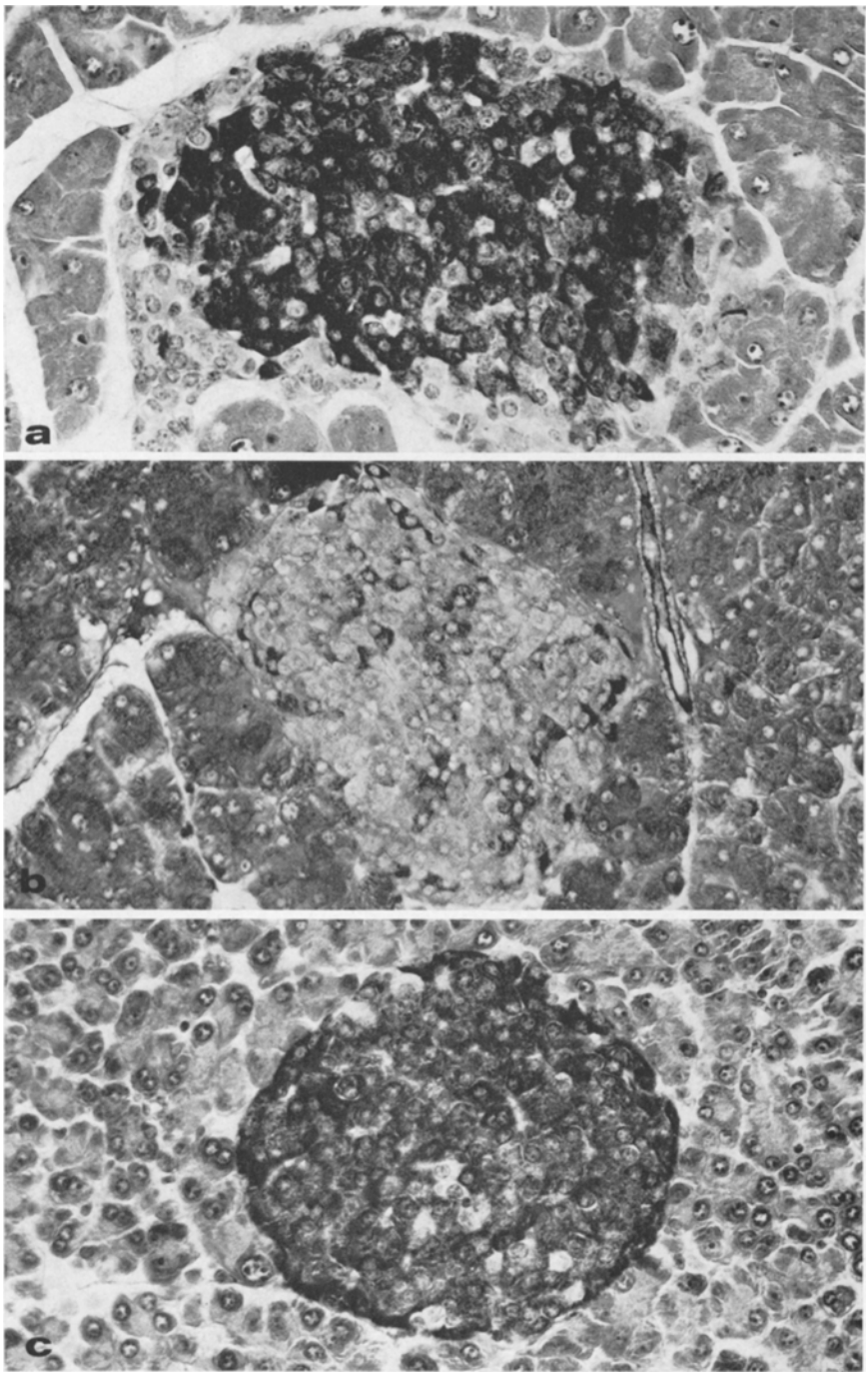

Fig. 7a—c. Pancreatic islets of mice. ×300. (a) Well-granulated B cells with strong AF intensity in normoglycemic controls. (b) Subtotally degranulated B cells with negative to weak AF intensity after 2 AIS injections (18 h after beginning of experiment). (c) Markedly regranulated B cells of moderate to strong AF intensity after 2 AIS and subsequent diazoxide injections (18 h after beginning of experiment)

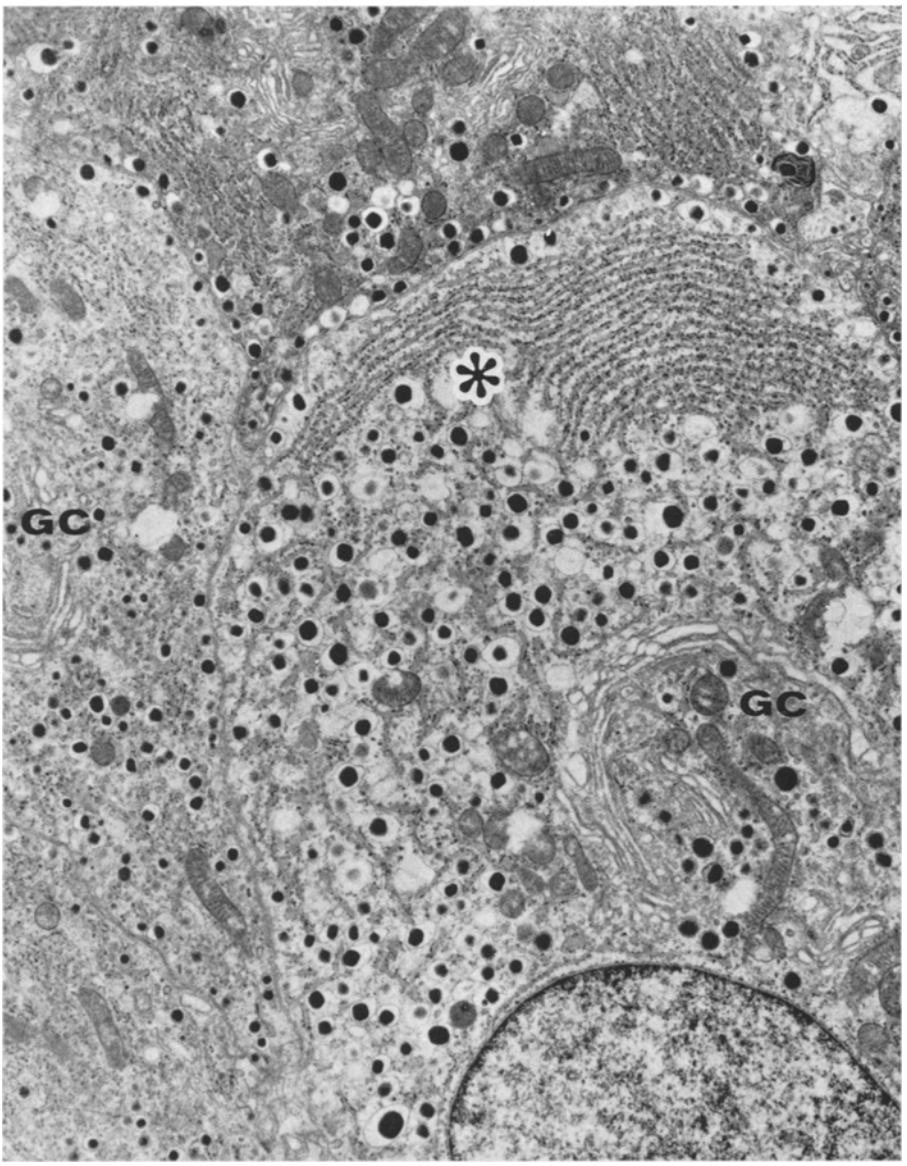

Fig. 8. B-cells after 2 AIS injections and subsequent diazoxide or DPH injections (18 h after beginning of experiment): strongly activated biosynthesis apparatus with hypertrophy and hyperplasia of the RER (asterix), hypertrophy of the Golgi complexes (GC), and marked new formation of secretory granules . × 19,300

cells was restored much earlier after AIS and subsequent diazoxide or DPH application than after AIS treatment alone (Fig. 6). Ultrastructurally, the regranulation phase in the AIS and diazoxide or DPH-treated animals was characterized by an unmitigated intense activation of the biosynthesis apparatus with an increased

formation of new secretory granules (Fig. 7). Occasionally granulolytic figures were observed.

Discussion

The exhibition of diazoxide to the animals results in a rapid and reversible increase in blood glucose. The degree and duration of the hyperglycemic syndrome are strongly dose-correlated (unpublished observation). As to the origin of the hyperglycemic effect of diazoxide it is suggested (a) that diazoxide may exercise an influence on the glucose metabolism via glycogenolysis, gluconeogenesis, and lipolysis (Nabwangu et al., 1965; Schultz et al., 1966; Jansen et al., 1967; Frerichs et al., 1968; Walfish et al., 1970), (b) that diazoxide may trigger a reactive catecholamine release with subsequent inhibition of insulin secretion (Wolff et al., 1963; Kvam et al., 1964; Meng et al., 1965; Staquet et al., 1965; Tabachnik et al., 1965; Raber et al., 1966; Zarday et al., 1966; Loubatières et al., 1967) and (c) that diazoxide may directly inhibit glucose-induced secretion of insulin, as was shown by in vitro examination (Loubatières et al., 1968; Basabe et al., 1970; Levin et al., 1972). In addition to the inhibition of the glucose-induced insulin secretion, diazoxide also suppresses the insulinotropic effects of glucagon and leucine, but not those of tolbutamide (Fajans et al., 1966). The mechanism of how diazoxide acts on insulin secretion is unknown. One possibility under discussion is that insulin secretion may be inhibited by inactivation of adenylcyclase and activation of phosphodiesterase with subsequent diminution of cAMP, which itself may play a role in the complex process of insulin release (Montague and Howell, 1973). As a second possibility it is thought that diazoxide influences insulin secretion via altered calcium handling by the B cells, since it has been shown to induce marked calcium efflux from the B cells (Malaisse et al., 1973). This finding is supported by results of recent histo- and ultracytochemical studies on calcium in B cells during diazoxide treatment which show a marked calcium depletion (Klöppel et al., 1975; Schäfer et al., 1975). Thus diazoxide probably acts on insulin secretion by changing the cytosolic calcium concentration which is thought to trigger the secretory process (Malaisse et al., 1973).

Creutzfeldt et al. (1969) found signs of marked crinophagy, an intracellular lysosomal digestion of secretory granules (Smith and Farquhar, 1966) in B cells of rats treated with diazoxide. These findings were regarded as the morphologic equivalent of inhibited insulin release. The present investigation confirms these findings. Remarkably, the inhibition of insulin release, which is recognizable histologically by hypergranulation, and ultrastructurally by inactivated secretory apparatus in addition to crinophagy, is maintained over a long period of time, despite high plasma glucose values.

DPH, as does diazoxide, inhibits the release of glucose-stimulated insulin release in vivo (Fariss et al., 1971; Malherbe et al., 1972; Levin et al., 1972) and in vitro (Kizer et al., 1970; Levin et al., 1970; 1972). Contrary to diazoxide, DPH is able to abolish the insulinotropic effect of sulfonylureas. It is suggested that DPH, by reducing the intracellular sodium concentration, changes the sodium-dependent calcium influx into the B cells and thus influences insulin secretion (Kizer et al., 1970; Bihler and Sawh, 1971). As recent studies on morphologic calcium demonstration in B cells reveal the same calcium depletion as seen after diazoxide treatment (Klöppel et al., 1975; Schäfer et al., 1975), this can be regarded as an

indirect reference to the hypothesis that DPH also alters insulin secretion via calcium metabolism of the B cells.

The histologic and ultrastructural features of B cells inactivated by DPH and diazoxide resemble each other closely. Thus, DPH application also accounts for distinct crinophagy in B cells. Nevertheless the granulolytic processes in B cells of DPH-treated animals seem to be less frequently found and less marked than those seen after diazoxide treatment.

The intracellular lysosomal digestion of secretory granules is considered to be a response of the cell to an intracellular accumulation of secretory products following a shutdown of secretion (Smith and Farquhar, 1966; Orci et al., 1970). Findings in the B cells of diazoxide or DPH-treated animals can also be interpreted in this way. The demonstration of strong activity of acid phosphatase, an enzyme regarded as a marker enzyme of lysosomes (Novikoff, 1963), within multigranulated bodies and dense bodies containing secretory granule cores supports a lysosomal nature of these organelles. Interestingly, the inhibition of insulin secretion during hypoglycemia due to the injection of exogenous insulin does not seem to induce an increase in crinophagic processes in B cells (Logothetopoulos, 1966; Schäfer und Klöppel, 1974). From this it can be concluded that an inhibition of insulin secretion does not necessarily result in an accelerated dissolution of secretory granules. The reason for these different responses of the B cell to inactivation of secretion is not known. As tentative explanations it may be suggested that crinophagy is only induced in those B cells (1) in which, in opposition to insulin release, insulin biosynthesis is not or only slightly inhibited, as seen under diazoxide or DPH treatment, and/or (2) in which electrostatic barriers between the surfaces of secretory granules are altered or reduced. The last possibility may be the consequence of a general calcium depletion of the B cell, as occurs under diazoxide or DPH treatment (Klöppel et al., 1975; Schäfer et al., 1975). In vitro, the fragility of a crude fraction of β granules exposed to the ionophore X-537 A can be diminished by an increase in the calcium concentration of the incubation medium (Hellman, 1975).

Until now the question of whether diazoxide has in addition an effect on insulin biosynthesis has been subject of only a few investigations (Basabe et al., 1970; Burr et al., 1971; Levin et al., 1972; Levy and Malaisse, 1975). No publications concerning this problem of the effects of DPH are available. An argument against a possible diazoxide-induced inhibition of insulin biosynthesis is the increased insulin content of the pancreas after diazoxide-inhibited insulin release (Basabe et al., 1970) and, in addition, the increased postinhibitory insulin release (Basabe et al., 1970; Burr et al., 1971; Levin et al., 1972). On the other hand, demonstration of a diminished proinsulin production after diazoxide (Levy and Malaisse, 1975) points to an inhibition of insulin biosynthesis. Present findings show that in mouse B cells, which had been, prior to a diazoxide injection, subtotally degranulated by double AIS injection, the biosynthetic apparatus is strongly activated in spite of inhibited insulin release. Thus regranulation occurs earlier in these B cells (within 12 h) than in those without diazoxide (within 18 h). Analogous results emerge for DPH treatment. As a consequence of these results we assume that diazoxide and DPH fail to inhibit strongly activated insulin

biosynthesis. In this in vivo examination with its long-lasting severe hyperglycemia, the inhibitory effect of diazoxide on proinsulin production, demonstrated by Levy and Malaisse (1975) in low glucose medium, is probably overcome by the maximum glucose effect on insulin biosynthesis. The suggestion that diazoxide seems to inhibit insulin biosynthesis only under conditions of normoglycemia or slight hyperglycemia might also explain the results of a study of Creutzfeldt et al. (1969) who found, under almost normoglycemic conditions, marked B cell degranulation and atrophy in rats treated with diazoxide over 14 days.

Considered together the results of the present investigation suggest that the B cells in DPH-treated mice show by analogy with the B cells in diazoxide-treated mice an increased lysosomal granulolysis activity, a fact which may be a characteristic for a certain mode of inhibition of insulin secretion. Furthermore, diazoxide and DPH are unable to prevent the regranulation of subtotally degranulated B cells during hyperglycemia. Hence, diazoxide and DPH do not seem to exert an inhibitory effect upon insulin biosynthesis under strong hyperglycemic conditions.

The authors wish to thank Mrs. Katrin Baack, Miss Monika Fischer, and Miss Ute Zeiger for their skilful assistance.

References

Basabe, J. C., Lopez, N. L., Viktora, J. K., Wolff, F. W.: Studies of insulin secretion in the perfused rat pancreas. Effect of diazoxide and AO 25. Diabetes **19**, 271–281 (1970)

Bihler, J., Sawh, P. C.: Effects of diphenylhydantoin on the transport of Na^+ and K^+ and the regulation of sugar transport in muscle in vitro. Biochim. biophys. Acta (Amst.) **249**, 240–251

Burr, I. M., Marliss, E. B., Stauffacher, W., Renold, A. E.: Diazoxide effects on biphasic insulin release: "Adrenergic" suppression and enhancement in the perifused rat pancreas. J. clin. Invest. **50**, 1444–1450 (1971)

Creutzfeldt, W., Creutzfeldt, C., Frerichs, H., Perings, E., Sikinger, K.: The morphological substrate of the inhibition of insulin secretion by diazoxide. Horm. Metab. Res. **1**, 53–64 (1969)

Ericsson, J.L. E., Trump, B. F.: Observations on the application of electron microscopy of the lead phosphate technique for the demonstration of acidphosphatase. Histochemie **4**, 470–87 (1964)

Fajans, S. S., Floyd, J. C., Knopf, R., Rull, J., Guntsche, E., Conn, J. W.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man J. clin. Invest. **45**, 481–92 (1966)

Fariss, B. L., Lutcher, C. L.: Diphenylhydantoin-induced hypoglycemia and impaired insulin release: effect of dosage. Diabetes **20**, 177–181 (1971)

Frerichs, H., Creutzfeldt, C., Creutzfeldt, W.: Inhibitors of insulin secretion. Acta diabet. lat. **5** (Suppl 1), 105–123 (1968)

Graber, A., Porte, D., Williams, R.: Clinical use of diazoxide and the mechanism for its hypoglycemic effects. Diabetes **15**, 143–48 (1966)

Hellman, B.: The effects of calcium and divalent ionophores on the stability of isolated insulin secretory granules. FEBS Letters **54**, 343–346 (1975)

Jansen, G. R., Hutchison, C. F., Zanetti, M. E.: Effect of diazoxide on glucose 'U-C-14 utilization in mice. Diabetes **16**, 777–783 (1967)

Kizer, J. S., Vargas-Cordon, M., Brendel, K., Bressler, R.: The in vitro inhibition of insulin secretion by diphenylhydantoin. J. clin. Invest. **49**, 1942–1948 (1970)

Klöppel, G., Bommer, G., Schäfer, H.-J.: Die Morphologie der B. Zelle des Pankreas unter dem Einfluß von Diazoxid und Diphenylhydantoin. Verh. dtsch. Ges. Path. **59**, 451 (1975)

Kvam, D. C., Stanton, H. C.: Studies on diazoxide hyperglycemia. Diabetes **13**, 639–644 (1964)

Levin, S. R., Booker, J., Smith, D. F., Grodsky, G. M.: Inhibition of insulin secretion by diphenylhydantoin in the isolated perfused pancreas. J. clin. Endocr. **30**, 400–401 (1970)

Levin, S. R., Grodsky, G. M., Hagaura, R., Smith, D.: Comparison of the inhibitory effects of diphenylhydantoin and diazoxide upon insulin secretion from the isolated perfused pancreas. Diabetes **21**, 856–862 (1972)

Levy, J., Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release XVII. Effects of sulfonylureas and diazoxide on insular biosynthetic activity. Biochem. Pharmacol. **24**, 235–239 (1975)

Logothetopoulos, J.: Electron microscopy of the pancreatic islets of the rat. Effects of prolonged insulin injections. Diabetes **15**, 823–829 (1966)

Loubatières, A., Mariani, M. M., Alric, R.: The action of diazoxide on insulin secretion, medullo-adrenal secretion, and the liberation of catecholamines. Ann. N.Y. Acad. Sci. **150**, 226–241 (1968)

Loubatières, A., Mariani, M. M., Alric, R., Ribes, G., Malbosc, H. de, Chapal, J.: Mécanisme d'action d'un sulfonamide hyperglycémiant le diazoxide. Ann. Endocr. (Paris) **27**, 787–794 (1967)

Malaisse, W. J., Pipeleers, D. G., Mahy, M.: The stimulus-secretion coupling of glucose-induced insulin release. XII. Effects of diazoxide and gliclazide upon ^{45}calcium efflux from perifused islets. Diabetologia **9**, 1–5 (1973).

Malherbe, C., Burrill, K. C., Levin, S. R., Karam, J. H., Forsham, P. H.: Effect of diphenylhydantoin on insulin secretion in man. New Engl. J. Med. **286**, 339–342 (1972)

Meng, K., Kroneberg, G.: Untersuchungen an der Ratte zur Frage der diabetogenen Wirkung von Saluretica. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **251**, 433–444 (1965)

Montague, W., Howell, S. L.: The mode of action of adenosine 3′5′-cyclic monophosphate in mammalian islets of Langerhans. Effect of insulin secretagogues on islet-cell protein kinase activity. Biochem. J. **134**, 321–327 (1973)

Nabwangu, Y., Staquet, M., Viktora, J., Yabo, R., Wolff, F.: Hormonal regulation of hyperglycemia due to diazoxide. Clin. Res. **13**, 246 (1965)

Novikoff, A. B.: Lysosomes in the physiology and pathology of cells. Contribution of staining methods. In: Lysosomes, pp. 36–73 (A. V. S. de Reuck, P. Cameron, eds.). London: J. and A. Churchill Ltd. 1963

Orci, L., Stauffacher, W. E., Dulin, A. E., Renold, A. E., Rouiller, Ch.: Ultrastructural changes in A-cells exposed to diabetic hyperglycemia. Observations made on pancreas of Chinese Hamsters. Diabetologia **6**, 199–206 (1970)

Schäfer, H.-J., Klöppel, G.: The significance of calcium in insulin secretion. Ultrastructural studies on identification and localization of calcium in activated and inactivated B cells of mice. Virchows Arch. A Path. Anat. and Histol. **362**, 231–245 (1974)

Schäfer, H.-J., Klöppel, G., Bommer, G.: Ultrastructural changes and histo-cytochemical cation patterns of the B cells associated with the action of diazoxide and diphenylhydantoin. Diabetologia **11**, 373 (abstract 238) (1975)

Schultz, G., Senft, G., Losert, W., Sitt, R.: Biochemische Grundlagen der Diazoxid-Hyperglykämie. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **253**, 372–387 (1966)

Senft, G., Sitt, R., Losert, W., Schultz, G., Hoffmann, M.: Hemmung der Insulininkretion durch α-Rezeptoren stimulierende Substanzen. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. **260**, 309–323 (1968)

Smith, R. E., Farquhar, M. G.: Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. J. Cell. Biol. **31**, 319–347 (1966)

Staquet, M., Yabo, R., Victoria, J., Wolff, F. W.: An adrenergic mechanism for hyperglycemia induced by diazoxide. Metabolism **14**, 1000–1009 (1965)

Tabachnick, I. I. A., Gulbenkian, A., Seidman, F.: Further studies on the metabolic effect of diazoxide. J. Pharmacol. exp. Ther. **150**, 455–467 (1965)

Walfish, P. G., Natale, R., Chang, C.: Beta adrenergic receptor mechanisms in the metabolic effects of diazoxide in fasted rats. Diabetes **19**, 228–233 (1970)

Wolff, F. W., Parmley, W. W.: Aetiological factors in benzothiadiazine hyperglycemia. Lancet **1963 II**, 69

Zaharko, D. S., Beck, L. V.: Studies of a simplified plasma insulin immunoassay using cellulose powder. Diabetes **17**, 444–457 (1968)

Zarday, Z., Wolff, V. J.: The effect of Diazoxide on catecholamines. Metabolism **15**, 257 (1966)

Dr. med. G. Klöppel
Pathologisches Institut
der Universität
Martinistr. 52
D-2000 Hamburg 20
Federal Republic of Germany