THE ENDOCRINE PANCREAS
IN THE CHINESE HAMSTER

STUDIES ON NON-DIABETIC, ALLOXAN-TREATED,
ZINC-DEFICIENT, AND SPONTANEOUSLY
DIABETIC ANIMALS

AKADEMISK AVHANDLING
SOM MED VEDERBÖRLIGT TILLSTÅND AV
MEDICINSKA FAKULTETEN VID UMEÅ UNIVERSITETET
FÖR VINNANDE AV MEDICINE DOKTORSGRAD
FRAMLÄGGES FÖR OFFENTLIG GRANSKNING
I VÄSTERBOTTENS LÄNS LANDSTINGS SJUKSKÖTERSKESKOLAS AULA
FREDAGEN DEN 14 MARS 1969 KL. 9.00 f. m.

AV

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Med. lic.

UMEÅ 1969
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STUDIES ON NON-DIABETIC, ALLOXAN-TREATED,
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BY

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This work was supported by grants from the Swedish Medical Research Council (Projects No. U 436, 12X-718-01, K67-12X-718-02, K68-12X-718-03, and B69-12X-718-4A) and the Medical Faculty, University of Umeå

Tryckeriaktiebolaget City

Umeå 1969
This thesis is based on the following publications:


VI. **Boquist, L.**: Cilia in normal and regenerating islet tissue. An ultrastructural study in the Chinese hamster with particular reference to the β-cells and the ductular epithelium. Z. Zellforsch. 89, 519—532 (1968).


These papers will be referred to by their Roman numerals.
Introduction

The study of small laboratory rodents exhibiting many, if not all, of the features of human diabetes makes an important part of current diabetes research. The information obtained in this way may supply us with some of the components necessary for the understanding of the pathogenesis of human diabetes mellitus (Renold, 1968). At least 13 mutations, inbred strains, and species lines that show a tendency to the development of diabetes and/or obesity are presently known (Renold & Dulin, 1967; Renold, 1968). Among these, mice with the obese-hyperglycemic syndrome have so far been most extensively utilized (cf. Westman, 1968). Another of these animals is the Chinese hamster in which the occurrence of spontaneous hereditary diabetes mellitus was first reported by Meier & Yerganian (1959). Since that time the Chinese hamster has been used in diabetes research, though a decreased incidence of diabetes in some colonies has hampered more extensive investigations. Another obstacle to the use of this species in laboratory studies is that the breeding has proved to be difficult, a fact that has led to the elaboration of various breeding methods (Yerganian, 1958; van Gaalen, 1963; Belčić & Weihe, 1967; Avery, 1968). By contrast with the other animals mentioned above obesity is not associated with diabetes in the Chinese hamster and hyperplasia of its islet β-cells does not seem to occur (Renold, 1968). Indeed, it has been stated that diabetes in this species in characterized by a decreased number of islets and a decreased β-cell mass (Carpenter et al., 1967; Sirek, 1969). Opposite to the findings in the sand rat, a high caloric diet does not precipitate diabetes in the Chinese hamster (Sims & Landau, 1967).

Since spontaneous diabetes mellitus in the Chinese hamster has been stated to be of pancreatic origin (Yerganian, 1964; Carpenter et al., 1967; Gerritsen & Dulin, 1967) studies on its endocrine pancreas would be of major importance. Among previous investi-
gations on this subject, those concerned with the morphology in non-diabetic hamsters are scanty and there is only one preliminary ultrastructural report on the morphology in diabetic hamsters (Luse et al., 1967). Systematic examinations of the effects of alloxan administration do not seem to have been performed previously in this species, nor are there any reports on trials to induce diabetic symptoms by means of dietary zinc deficiency.

Against this background the aims of the present work can be summarized as follows:

1. To characterize in more detail the non-diabetic Chinese hamster with respect to a) the morphology of its pancreatic islets, b) the functional capacity of its endocrine pancreas as reflected in the blood glucose regulation, and c) the glutathione content of its tissues.

2. To test the possibility of provoking morphologic alterations and a defective function of the β-cells in apparently normal animals by exogenous means such as a) alloxan or b) a zinc deficient diet.

3. To characterize the spontaneously diabetic Chinese hamster with respect to the morphology of its pancreatic islets as compared to that of normal and alloxan-treated animals.
Materials and Methods

The materials and methods used will only be briefly described here and the reader is referred to the original articles for details.

Animals
Non-diabetic (I—VI and VIII) and diabetic (VII) Chinese hamsters of both sexes were used. They belonged to two inbred stocks kept at the Institute of Pathology in Umeå since 1964. Both these stocks emanated originally from Dr. Yerganian’s colony in Boston, U.S.A. Breeding was performed according to the description given by Yerganian (1958). The animals were fed a conventional diet for laboratory rodents (I—VII) or a zinc-deficient diet (VIII).

Light Microscopy
For fixation, 10 % formalin or Bouin’s fluid were most often used. The following histological and histochemical methods were most frequently applied: van Gieson’s stain, aldehyde fuchsin as modified by Maske (1955), chrome alum hematoxylin, the modified Davenport method (Hellerström & Hellman, 1960), silver impregnation according to Grimelius (1968), the pseudoisocyanin procedure (Schiebler & Schiessler, 1959), and the sulfide-silver method (Voigt, 1959).

Electron Microscopy
The pancreatic specimens were usually fixed by immersion in 1 % osmium tetroxide in veronal acetate buffer, or in 2.5 % glutaraldehyde in phosphate buffer, followed by postfixation in osmium tetroxide. Epon was used for embedding and the sections were cut on an LKB Ultrotome III. After staining with uranyl acetate and
lead citrate the sections were examined in a Siemens Elmiskop I A and a Zeiss EM 9.

**Determinations of Glucose, Glutathione, and Insulin**

For detection of glucosuria glucose oxidase strips were used. Blood was sampled under light ether anesthesia from the orbital venous plexus. Blood glucose was assayed by the glucose oxidase method. The glucose tolerance was investigated after intraperitoneal or intravenous administration of glucose at a dose of 2 g/kg body weight. Glutathione was determined by the nitroprusside (*Falkmer, 1961*) and the o-phthalaldehyde (*Havu, 1969*) methods. The level of immunoreactive insulin in serum was assayed with the double antibody radioimmunological technique according to *Hales & Randle* (1963).
Results and Discussion

Morphology of the Endocrine Pancreas (I and II)

Four different types of islet cells were observed in the Chinese hamster. By light and electron microscopic criteria they were classified as corresponding to the $\beta$-, $\alpha_1$-, $\alpha_2$-, and agranular cells of other mammals, including man (Björkman et al., 1966; Hellman, 1966; Hellman & Hellerström, 1969). The islet cells showed similar ultrastructural appearance irrespective of whether the specimens were fixed in osmium tetroxide, or in glutaraldehyde followed by postfixation in osmium tetroxide.

The $\beta$-cells are well known to produce insulin. The $\alpha_2$-cells are generally considered to produce glucagon (Hellerström & Hellman, 1962; Petersson & Hellman, 1963; Unger et al., 1967). In most species the $\alpha_2$-cells are easily identified. In some cases there are reports on morphological variations among them. Björkman et al. (1963 and 1966) found, for example, light and dark $\alpha_2$-cells in the horse and in the human fetus. Such variations were not seen in the Chinese hamster.

As to the existence and function of other islet cell types there are different opinions. The $\alpha_1$-cells are characterized by an argyrophil reaction in the modified Davenport method (Hellerström & Hellman, 1960). It has been said that these argyrophil cells correspond to what has been denoted as D-cells (cf. Epple, 1968). Since not only the $\alpha_1$-cells, but also the agranular cells show cytoplasmic staining properties (e.g. affinity for light green) that are considered characteristic of the D-cells, it has been argued that the D-cell concept comprises both $\alpha_1$-cells and agranular cells (cf. Björkman et al., 1966; Hellman & Hellerström, 1969). Hence, the concepts of $\alpha_1$-cells and D-cells are not congruent. The results of the present study support the latter opinion, since the green stained cells (PAS-
trichrome staining) had a localization somewhat different from that of the $\alpha_1$-cells. The function of the argyrophil cells ($\alpha_1$-cells) is not known. They have been supposed to produce gastrin or a fat regulating hormone (cf. Epple, 1968; Fujita, 1968). Furthermore, these cells have been thought to be precursors to $\beta$-cells (Legg, 1967) or amphiphil cells (Epple, 1968), or to be modified $\alpha$-($\alpha_2$-)cells (Like, 1967). The relative contribution of $\alpha_1$-cells to the islet volume has been suggested to be correlated to the serum glucose level (Westman, 1968).

The agranular cells have been found to be abundant in some lower vertebrates where they have been suggested to represent immature precursor cells to the granulated cells (Falkmer et al., 1964; Falkmer & Bergdahl, 1967). So far no evidence for the production of any hormone by these cells has been obtained (Blair et al., 1969). In the mammalian pancreatic islets a small proportion of agranular cells seems to be present (Hellman & Hellerström, 1969). In the light microscopic procedures (notably the granule stainings) applied in the present work, a few cells without characteristic features were found. At least some of these cells might be of the agranular type. Because of their lack of distinguishing light microscopic features, it was not possible to include the agranular cells in the estimation of the cell ratios.

The proper identification of the agranular cell type has only been possible in the electron microscope. This cell type has been found to be ultrastructurally characterized by low cytoplasmic electron density, no or almost no secretory granules, low or moderate amount of mitochondria, as well as rather inconspicuous Golgi complex and endoplasmic reticulum. The concept of the agranular cells as precursor cells (Falkmer et al., 1964) has been used as a working hypothesis in the course of the present work and will be used as such in further studies. In non-diabetic hamsters there were cells with prominent Golgi complex and endoplasmic reticulum which because of a sparse occurrence of secretory granules (sparsely granulated cells) seemed to be related to the agranular cells. This may be consistent with the working hypothesis since the sparsely granulated cells may represent an intermediate stage in the evolution from agranular to granular cells. The assumption that "intermediate cells" occur makes the distinction between these cells and
degranulated cells difficult. It seems, however, that the cytoplasmic electron density is lower in the "intermediate cells" than in the degranulated cells.

Blood Glucose Regulation and Glutathione Content (III)

Blood sampling under light ether anesthesia by puncture of the orbital venous plexus has been employed in other studies on Chinese hamsters (cf. Sirek, 1969) and mice (Westman, 1968). Handling of the animals and ether anesthesia might well to thought to give rise to blood glucose alterations. In an attempt to see whether the blood glucose level was affected by the repeated sampling or by the light ether anesthesia employed in the present study, no obvious blood glucose alterations were recorded. The blood glucose level in non-fasting, non-diabetic Chinese hamsters as determined in 88 animals was 107 ± 1.2 mg/100 ml (mean value ± S.E.). The upper limit for hypoglycemia has in the present work been 80 mg/100 ml and the lower limit for hyperglycemia 135 mg/100 ml. By slight hyperglycemia has been meant values between 135—180 mg/100 ml, and by moderate hyperglycemia values between 180—300 mg/100 ml. Higher values have been designated as marked (or synonyms) hyperglycemia.

The glucose tolerance curve for non-diabetic animals showed a peak value of about 185 mg/100 ml at 30 minutes. Transient hyperglycemia was evoked by the injection of glucagon (0.2 mg/kg), adrenalin (100 µg/kg), hydrocortisone (50 mg/kg), and cobaltous chloride (25—100 mg/kg), whereas growth hormone (2 IU/animal /day) had no effect on the blood glucose level. Subtotal pancreatectomy induced a moderate hyperglycemia in most animals. While the spontaneously diabetic Chinese hamster has been said to require large amounts of insulin for control (Gundersen et al., 1967), the non-diabetic animals were found to be sensitive to even fairly small doses of ox insulin in the present work. As far as can be concluded from these results, the blood glucose regulation of non-diabetic Chinese hamsters shows no conspicuous abnormalities as compared with that of other mammals, including man.

The glutathione content in the non-diabetic Chinese hamsters was found to be within the normal range for many species. For example,
the mean value of this compound was about 35 mg/100 ml in blood and 124 mg/100 ml in liver. The corresponding values for rats are 34 and 112 (Grunert & Phillips, 1951). The glutathione level in microdissected pancreatic islets after the administration of diabetogenic compounds (e.g. alloxan), in dietary experiments, as well as in spontaneously diabetic hamsters will be the subject of a forthcoming work.

**Effects of Alloxan Administration** (I, III, IV and V)

Alloxan administration evoked selective necrosis of the islet $\beta$-cells, hyperglycemia, and glucosuria, indicating that the Chinese hamster, like most other species (cf. Schmidt, 1967), is sensitive to alloxan. The resistance of the guinea pig to alloxan has been suggested to be due to the low content or absence of zinc in its islets (Maske & Weinges, 1957) or to the high concentration of glutathione in its blood (cf. Scherer, 1955). If this holds true, it seems to be natural that the Chinese hamster belongs to the alloxan sensitive animals since zinc appeared to be present in its pancreatic islets (I and II) and since there was no exceptionally high concentration of glutathione in its parenchymal organs or blood. It has been found that mice injected with small amounts or $^{14}$C-2-alloxan show a high radioactivity in the pancreatic islets as compared with that in other tissues (Hammarström & Ullberg, 1966; Hammarström et al., 1967). The basis for the selective effect of alloxan on the $\beta$-cells is, however, incompletely known.

**Islet Cell Regeneration** (IV and V)

In the course of the present work it was found that regenerative phenomena occurred in alloxan-treated animals. The regeneration seemed to take place from ductules (tubular structures) containing cells with a cytoplasm of low electron density (clear cells). These cells were furthermore often devoid of secretory granules and were reminding of the agranular islet cells. Support for the role of the ductules and the clear cells in the regeneration was that a) some ductule cells were aldehyde-fuchsinophil in the light microscope, and exhibited particles reminiscent of secretory granules in the electron microscope, and b) buds or small islets of clear cells or
granulated cells occurred in connection with or close to ductules. Similar observations have been made in duct ligated and alloxan diabetic rats (*Zweens & Bouman*, 1967).

Within the islets of alloxan-treated hamsters there were also a great number of cells without obvious granulation (clear cells). It was thought that some of these cells were of the agranular type described above for the non-diabetic animals, whereas others were degranulated β-cells. The clear cells in the islets were numerous the first few days after the administration of alloxan. This might indicate a degranulation of β-cells and/or a proliferation of agranular cells. The subsequently decreasing amount of clear cells in the islets can be explained by a new formation of secretory granules in agranular cells and/or in degranulated β-cells. In addition, there might well be a restitution and proliferation of only moderately damaged β-cells. Any signs of "acino-insular transformation" (*cf. Patent & Alfert*, 1967) were not recorded. On the basis of these observations, a hypothesis for future work (see above) was formulated, implying that agranular cells in ductules and islets are potential loci of cell renewal.

The pancreatic islets in non-diabetic Chinese hamsters obviously have a great regenerative capacity conforming to that of guinea pigs (*Hausberger & Ramsay*, 1955). Furthermore, as manifested by the islet hyperplasia observed in, for example, obese-hyperglycemic mice (*cf. Westman*, 1968) a marked power for neoformation also characterizes at least some strains of mice.

**Ciliated Pancreatic Cells** (VI)

Though cilia occasionally have been observed in the pancreatic islets of some species, there are no systematic investigations on this subject. The significance of these structures in endocrine cells is not known. In the present study cilia occurred in non-diabetic and alloxan-treated (VI), as well as in spontaneously diabetic (VII) animals. So far, cilia have only been found in ductular epithelium and in β-cells. In the alloxan-treated hamsters cilia were more numerous in the regenerative period, indicating a relationship to cell renewal. This may denote that cilia in endocrine cells are rudimentary structures without any function, mainly occurring in newly formed cells. It can, however, be speculated that they have
some significance for cell renewal. Cilia have been observed in association with amitotic cell division in endocrine cells (Pehlemann, 1968). Though mitoses have been found in the islets of mice (Hellerström et al., 1962; Logothetopoulos & Brosky, 1968), and humans (Potvliege et al., 1963), as well as in the presently investigated alloxan-treated animals, there may, in addition, well be amitotic cell division in which cilia may be involved. Cilia have been assumed to develop following centriole division with the production of numerous basal bodies which migrate from the perinuclear area and make contact with the cell surface (Hilding & Hilding, 1966; Milhaud & Pappas, 1968). On the other hand, it has been suggested that cilia develop when the basal bodies are dispersed throughout the cytoplasm and that a vesicle is involved in this process (Sorokin, 1968; Martinez Martinez & Daems, 1968). The findings in the present study are consistent at least with the latter of these opinions.

**Effects of Dietary Zinc Deficiency (VIII)**

According to the "zinc-theory" (Okamoto, 1949) the concentration of zinc in the islets plays a role for the pathogenesis of alloxan diabetes and possibly also for that of spontaneous diabetes. Zinc deficiency has been reported to induce impaired glucose tolerance in rats (Hove et al., 1937; Quarterman et al., 1966). In the present study Chinese hamsters were fed a zinc deficient diet. Decreased concentration of zinc was found in the testes. Hence, there was an effect of the diet in males, and since sex differences were not recorded in the other investigations of this study it is probable that there was an effect of the diet also in females. The zinc deficient animals showed no glucosuria or hyperglycemia but the intraperitoneal and intravenous glucose tolerance was impaired. The serum immunoreactive insulin level of the zinc deficient hamsters did not deviate from that of control animals and there was no significant increase of this level after glucose injection. The plasma insulin level in spontaneously diabetic Chinese hamsters and in human diabetics has also been said to be unchanged after glucose administration (Gerritsen & Dulin, 1967). The β-cells of the zinc deficient hamsters exhibited decreased granulation and weak pseudoisocyanin reaction. Since the β-cell granules generally are
considered to contain insulin (Hartroft & Wrenshall, 1955; Hjertén et al., 1964; Logothetopoulos et al., 1965) and since the intensity of the pseudoisocyanin reaction has been regarded to reflect the content of insulin in the β-cells (Schiebler & Schiessler, 1959; Coalson, 1966), it appeared that the content of insulin in the islets of zinc deficient hamsters was decreased. Zinc has been suggested to play a role for the storage and secretion of insulin (Maske, 1957; Yoshinaga, 1968). Hence, it is possible that the decreased insulin content in the β-cells of the zinc deficient animals might be due to diminished amount of zinc. The zinc content in the islets of hamsters with spontaneous diabetes will be separately investigated.

*The Endocrine Pancreas in Spontaneous Diabetes Mellitus*  
(VII)

Obese-hyperglycemic mice have been found to show raised blood glucose level from 1 month of age and return of this level to the normal at 6 months of age (Westman, 1968). Decreased blood glucose concentration with increasing age has also been observed in diabetes in the Wellesley hybride mouse (Like & Jones, 1967). Early onset of diabetic symptoms and increasing severity with age occurs in "mutation diabetes" in mice (Coleman & Hummel, 1967). In the Chinese hamster early onset (18 days to approximately 2 months) of diabetes has been reported (Meier & Yerganian, 1959; Gundersen et al., 1967; Malaisse et al., 1967). Among the presently investigated hamsters there were also those with early onset of diabetic symptoms. The duration of diabetes in these animals varied from 1 to 19 months. The course of diabetes in the Chinese hamster has been found to be either stable, or fluctuating, either gradually worse, or definitely better with time (Gundersen et al., 1967). It has been reported that the blood glucose level of diabetic and non-diabetic hamsters is not influenced by sex, age, duration of diabetes, age at onset of diabetes, diet or insulin therapy (Gerritsen & Dulin, 1967). In the present study the highest blood glucose values at the time of sacrifice were seen in animals with diabetes of short duration. Among these animals nuclear polymorphism and pyknosis, as well as necrotic changes of the β-cells were seen. The other morphological alterations showed no clear relation to the duration of the disease. The β-cells of
diabetic hamsters often exhibited decreased granulation, glycogen infiltration, and accumulation of electron lucent material. Various cytoplasmic bodies, probably of degenerative nature, occurred in the \( \beta \)-cells. Islet regeneration and hyperplasia have been observed in human diabetics, but the newly formed islets have been said to be functionally deficient and devoid or almost so of \( \beta \)-cells (cf. Theodossiou, 1956). Cells resembling the agranular ones seemed to be rather numerous in the diabetic Chinese hamsters studied by Luse et al. (1967). In the present study there were, however, no conspicuous signs of regeneration of the kinds seen after alloxan administration. This accords with the observation of decreased \( \beta \)-cell mass in diabetic hamsters (Carpenter et al., 1967). Though the pathogenetic mechanism still is poorly understood, the present findings indicate that spontaneous diabetes mellitus in the Chinese hamsters is due to decreased function and deficient regenerative capacity of the \( \beta \)-cells.
General Summary and Conclusions

1. The following main observations on non-diabetic Chinese hamsters were made:
   a) The pancreatic islets were found to be composed of \(\beta\), \(\alpha_2\), \(\alpha_1\), and agranular cell types. Heavy metal compounds, probably zinc, were present in the islets.
   b) The fasting blood glucose level was 107 ± 1.2 (mean value ± S.E.). The glucose tolerance curve after intraperitoneal administration of 2 g/kg body weight of glucose showed a peak value of about 185 mg/100 ml at 30 minutes. Transient hyperglycemia was evoked by the injection of glucagon (0.2 mg/kg), adrenalin (100 \(\mu\)g/kg), hydrocortisone (50 mg/kg) or cobaltous chloride (25—100 mg/kg), whereas the administration of growth hormone (2 IU/animal/day) had no effect on the blood glucose level. The animals were sensitive to ox insulin and they showed a moderate hyperglycemia after subtotal pancreatectomy.
   c) The concentration of glutathione was moderate in the pancreas, myocardium, kidneys, and erythrocytes, and somewhat higher in the liver.

2. a) The Chinese hamster was sensitive to alloxan administration that evoked selective necrosis of the islet \(\beta\)-cells, glucosuria, and hyperglycemia. These alterations were of transitory nature, apparently due to regeneration of the \(\beta\)-cells. In the regenerating pancreatic parenchyma increased frequency of ciliated \(\beta\)-cells and ductular cells was found.
   b) Chinese hamsters fed a zinc deficient diet exhibited decreased glucose tolerance and signs of low insulin content in the islets, but there was neither glucosuria, or hyperglycemia, nor any alteration of the serum immunoreactive insulin level.
3. The β-cells in spontaneously diabetic Chinese hamsters often showed decreased granulation, glycogen infiltration, accumulation of electron lucent material, and various cytoplasmic bodies. Occasionally, the β-cells of these animals exhibited nuclear polymorphism, pyknosis, and necrotic changes. Signs of regeneration of the kinds seen after alloxan administration were not recorded.

Though the pathogenesis of the spontaneous diabetes mellitus in the Chinese hamster still is poorly understood, the present findings indicate that this kind of diabetes is due to decreased function and deficient regenerative capacity of the islet β-cells.
REFERENCES


