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Histochemical and immunocytochemical investigation of endocrine tissue of pancreas after administration of B-cytotoxic chemicals

Authors showed that intravenous injection amino acids Cystein and L-Hystidine prevent in majority of animals developing of experimental diabetes caused by chemicals formed in B-cells chelat complexes with Zn^{+2}-ions. It was shown that in all cases administration of these amino acids prior injection of diabetogenic chelator as Diphe nylthiocarbazon (Dithizon) accompanied by almost complete absence of binding of Zn^{+2}-ions in B-cells with formation of toxic complexes which result destruction and death of B-cells within short time. Authors suppose that protective effect is determined by presence in molecules of Cystein and L-Hystidine of SH-groups reacted with reacted with Zn^{+2}-ions in B-cells with forming of not toxic complexes and not able to forming of complexes with a Dithizon. Authors showed also that from the used various histochemical and immunocytohistochemical methods insulin staining in B-cell more precise results was obtained using of immunohistochemical and fluorescent Diethylpseudoisocyanine methods. However, Aldehyde-fuchsine method and method Victoria 4R more suitable for investigation state of histostructure of pancreatic islets.

Key words: pancreas, B-cells, Zn^{+2}-ions, Cystein, L-Hystidine.

Background

Okamoto K. showed that Diphenylthiocarbazone (Dithizon) possess ability for selective destruction of B-cells accompanied by developing of diabetes within a few days [1]. More later it was showed that injection of Alloxan, Derivatives of 8-oxyquinolin and Streptosotozin result selective death of B-cells too. Among methods for investigation result of action of these substances on islets histochemical and immunocytochemical methods have a few advantages as: 1)detail analysis of state of histostructure of islets; 2) analysis of insulin and Zn^{+2}-ions content and disposition of hormone in cytoplasm of B-cells; 3) reveal the early histological and histochemical changes in islets. Pancreatic B-cells contained a large amount of Zn^{+2}ions [2–4] as salivary glands and prostate. In B-cells Zn^{+2}-ions take part in processes of biosynthesis of insulin as in of storage by forming of Zn^{+2}-insulin complex [5, 6]. It is known that Zn^{+2}-ions in B-cells formed with insulin a deposited form of hormone as Zn^{+2}-insulin complex [5]. Proinsulin forms a zinc containing hexamer soon after its synthesis. In addition the zinc ions enhance proinsulin solubility and render insulin insoluble. Zinc ions also appear to play an important role in the microcrystalline character of the precipitated insulin granule [1]. Pancreas of rat, rabbit, dog, cat, some fish, human, birds, mice, hamster, porcine, hoerst, contained a large amount of Zn^{+2}-ions [1]. By electron histochemistry method it was showed that Zn^{+2}-ions concentrated in B-granules only contained deposited form of insulin [7] and that destruction of B-cells caused by Dithizon, which formed in B-cells toxic complexes with Zn^{+2}-ions, started by destruction of B-granules [8, 9].

Aim of work: to investigate influence of 2 groups of diabetogenic chemicals on histostructure and insulin content in B-cells of pancreatic islets: 1) diabetogenic zinc-binding substances as Diphenyldithiocarbazone (Dithizon) [DZ] and 8-para(toluenesulphonylamino)quinolin [8PTSQ]; 2) SH-contains aminoacids as Gluthation Reduced form, Cystein, Hystidine and Na salt of Diethyldithiocarbamic Acid [DDCA]; 3) to compare results of staining of islets by various histochemical and immunocytochemical methods as to compare results of action of diabetogenic substances on B-cells in experiences on animals and on isolated pancreatic islets.

Methods

Animals. 14 Rabbits 2240–3050 g, 22 Rats 158–175 g and 12 white mice 33–42 g were used. 1. Experiences with Dithizon. 2. Experiences with 8PTSQ. 3. Experiences with Na salt of Diethyldithiocarbamic Acid [DDCA]. 4. Experiences with Cystein and Hystidine.

Dithizon [DZ] as 8PTSQ possess a high chemical affinity for Zn^{+2}-ions and in vitro formed color complexes as Zn^{+2}-chelator [1, 2]. 8TSQ formed fluorescent green complexes with Zn^{+2}-ions visible using fluorescent microscopy and Dithizone formed red DZ-Zn^{+2}-ions complex visible using dark microscopy. Maximum of absorbance of Zn^{+2}-DZ complex on spectrum of absorbance correspond for 530 nm [3]. 8PTSQ is very sensitive for revealing of Zn^{+2}-ions in solutions contained minimal concentrations as 10^{-7}–10^{-8} of Zn^{+2}-ions and is used for color revealing of its in solutions. Diabetogenic properties of all these substances were established previously and determined by ability to form complex salt with Zn^{+2}-ions in cytoplasm of B-cells that result necrosis and death of cells within short time [4, 5].

Na salt of Diethyldithiocarbamic Acid [DDCA] possess a high affinity for Zn^{+2}-ions too [17]. DDCA formed not toxic for B-cells complex with Zn^{+2}-ions and not result developing of experimental diabetes [8]. Contrary, binding of Zn^{+2}-ions by DDCA, injected in dose of 1000 mg per kg body weight animals protect B-cells in 95 %-100 % animals of death and of developing of diabetes caused by DZ and diabetogenic derivatives of 8-oxyquinolin for 12–24h [5].

Preparing of solutions

Preparing of 8PTSQ solution: 25 mg. of 8PTSQ (Inst. High Pure Chemicals, Moscow) was dissolved in 65 % Ethanol on +70º Celsium and injected to Rabbits 35,5–38,8 mg/kg [9]. Preparing of DDCA solution: 1000 mg of DDCA (MERCK, Germany) dissolved in 10 ml of bidistillate.

Frozen sections of Rat's Pancreas 4 mcm were investigated 10 min past injection using dark microscopy. Intensity of staining was measured by photometer. 2nd part of pancreas tissue was fixed in Ethanol 70 % contained dissolved H_2S; paraffin sections of tissue were stained by 0,4 % acetone solution of 8PTSQ [6] and were investigated on fluorescent microscope.

Pancreas tissue was fixed in Bouin 24h. Staining technologies. Following methods were used for staining 4–5 mcm paraffin sections of pancreas.

Aldehyde-fucshine method by Gomori G. Violet granules in cytoplasm of B-cells correspond to deposited form of insulin [10–13].Intensity of color of cytoplasm of B-cells directly correspond to insulin content in cytoplasm [12–13]. Insulin content was calculated as parameter K=AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Diethylpseudoisocyanine fluorescent method. Schiebler T. and Schiessler S. showed that A chair of oxidized insulin reacted with Diethylpseudoisocyanine chloride with formation of red fluorescent complex which fluoresces in UV light 360–370 nm. We have used modernized by Coalson R.E. method [14–15].

This method method is used not often, that is why we offer the detailed description of staining procedures. Preparing of staining solution: 0,04 % water solution of Diethylpseudoisocyanine (SERVA, Germany). Staining procedures: 1) deparaffinization of sections in xylol; 2) alcohol 90º, 80º, 70º 1 min in each; 3) washing in cold water; 4) oxidation 0,5–2 min; oxidation solution: 5 ml of 5 % H_2SO_4 + 5 ml 2,5 % solution of $KMnO_4$ + 30 ml bidistillate at +28º Celsius; 5) washing in cold water; 6) 5 % solution of oxalic acid — 5 sec; 6) washing in 2 portions of cold water; 7) 0,04 % cold solution of Diethylpseudoisocyanine — 20 min in refrigerator at +4º Celsius; 8) washing in cold water 5 min; 9) store in refrigerator 1,5–3 h. Insulin content was calculated as parameter K = IF1/IF2 where: IF1 — intensity of fluorescence of intact B-cells; IF2 — intensity of fluorescence of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Victoria Blue 4r method staining of insulin (V4R), Diphenylnaphthylmetane, colour index 42563; MERCK, Germany; FERAK, West Berlin). It was showed by F.Wohlrab (16) that V4R in aqueous solution interacted with oxidized A-chair of insulin that is accompanied by painting of cytoplasm of B-cells in a blue color proportionally to the amount of insulin [16]. V4R paints some peptides hormones but B-cells produce insulin only. This method method is used not often, that is why we offer description of staining procedures. Staining procedures: 1) deparaffinization of sections; 2) washing in cold water a few min; 3) oxidation 3–5 min (oxidation solution: 0,3 % $KMnO_4$ 50 ml + 0,3 % H_2SO_4 50 ml; wash sections; 4) place sections in 2–5 % water solution of natrium bisulphate — 1 min; wash sections; 5) 70º alcohol — 1 min; 6) stain in staining solution (96º alcohol 100 ml + Victoria Blue 4R — 1 g) 15 min — 2h; wash sections; 7) stain in 0,5 %

water solution of Phloxine 30–120 sec.; wash sections; 8) 5 % water solution of phosphortungstic acid 1–2 min; wash section in water; 9) stain in 0,5 % water solution of Light Green 1–2 min; 10) dehydratation in 96 % alcohol. Method was adopted for using of sections of tissue culture of islets [17]. Insulin content was calculated as parameter K = AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Staining by Dithizon. Preparing of Dithizon solution: 30 mg of Dithizon, (SIGMA, USA) + 10 ml bidistillate + 0.2 ml 25 % NH_4OH 10 min. mixing on temperature +70º at Celsium. Solution was injected intravenously to Rabbits and to Mice 46–48,6 mg/kg.

Frozen sections of 4 mcm were investigated 5–10 min past injection on dark microscopy. Density of staining was measured using photometer. Insulin content was calculated as parameter K=AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Staining by 8PTSQ. Zn^{+2}–8PTSQ complex radiates intensive green fluorescence under UV-light 360–370 nm length of wave that was confirmed by spectral analysis [18–20]. Cytoplasm of B-cells not contained Cadmium. Past long time prolonging testing in Institute of High Pure Chemicals (Moscow) 8PTSQ was proposed as fluorescent reagent for identification of very small amounts of Zn^{+2} in solutions and tissues. Later by Y.A.Lasaris and coll. 8PTSQ was tested for revealing Zn^{+2}-ions. 8PTSQ is high specific reagent for staining of Zn^{+2}-ions in pancreatic B-cells. Frozen sections of rat's Pancreas 4 mcm were investigated on fluorescent microscope. Staining procedures: 1) staining by 0,4 % acetone solution of 8PTSQ; 3–4 drop of solution placed on section; wash section by 3 portions of bidistillate. Intensity of fluorescence was measured [21]. Insulin content was calculated as parameter K=AB1/AB2 where: IF1 — intensity of fluorescence of intact B-cells; IF2 — intensity of fluorescence of B-cells past action of diabetogenic substances (calculated as 1,00).

Immunofluorescent staining of insulin. Anticorps for insulin (Institute of Diabetes «Gerhardt Katsch») were used for staining sections of pancreas tissue.

Immunohistochemical method. Standard kits for insulin (DAKO, Denmark) were used for staining. Insulin content was calculated as parameter K=AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Isolation of pancreatic islets by Collagenase

Animals. Pancreas of 14 rats LEWIS 4–5 days old and 8–10 weeks old human embryons were used. Isolation procedures: dissected pancreas tissue were treated 3 times 3 min each by 2 % solution of Collagenase (Boehringer Mannheim, Germany; FLUKA, Switzerland); human embryons pancreas was treated by Collagenase 2 times 1 min each; rinse 3 times in cold Hanks solution and centrifugation; cultivation 12h at +37º Celsius in medium RPMI 1640 (SERVA, Germany) with bovine serum + 5.5 mM of Glucose, pH 7.32–7.41. Fixation in Bouin 15 min – 1 h and embedding in paraffin. Sections 4 mcm were used. Dithizon solution 0,4 ml was added in 10 ml of nutria media 199 contains islets for 20 min that correspond to concentration about 40 mg/kg in experiences on animals. Than media 199 was changed for new fresh portion + 5,5 mmol/l of Glucose + bovine serum; cultivation 5h at pH=7,34–7,41.

Results

Isolated pancreatic islets. Intact islets.

Aldehyde-fuchsine staining. Intact islets. Histostructure of islets without histological changes. Islets have oval form and contains deposited insulin (blue-violet color) (fig. 1.1). Insulin content: AB=1,93±0,06.

Victoria 4R staining. Histoctructure of islets without histological changes. Islets contains a large amount of deposited insulin (fig. 1.2). Insulin content: AB=1,97±0,05.

Immunohistochemistry. Histoctructure of islets without histological changes; B-cells contains a large amount of deposited insulin (fig. 1.3). Insulin content: AB=1,81±0,04.

Diethylpseudoisocyanine method. Histoctructure of islets without histological changes; Red fluorescence of A-chair of insulin; insulin content: IF=2,02±0,05.

Fluorescent staining of Zn^{+2}-ions. Intensive green fluorescence of B-cells (fig. 1.5): IF=2,08±0,05.

Past action of Dithizon

Aldehyde-fuchsine staining. Necrosis, destruction and death of B-cells; marked decreasing of insulin content in majority of B-cells (fig.1.6). Insulin content: AB=1,14±0,04.

Victoria 4R staining. Destruction of islets, destruction and death of B-cells; decreasing of insulin content in majority of B-cells (fig. 1.7): AB=1,38±0,05.

Immunohistochemistry. Deformation of islets; destruction and death of B-cells; decreasing of insulin content in majority of B-cells (fig.1.8): AB=1,18±0,04.

Diethylpseudoisocyanine staining. Destruction of islets; marked decreasing of insulin content in B-cells (fig. 1.9): IF=1,07±0,011.

Fluorescent staining of Zn^{+2}-ions. Almost complete disappearing of Zn^{+2}-ions from B-cells (fig. 1.10): IF=1,01±0,02.

Human embryo islets

Aldehyde-fuchsine staining. Intact embryo islets structure contains not compact or compact groups of polygonal B-cells not completed formation of islet; insulin content visually is reduced comparatively with B-cells of adult rats and rabbits (fig. 1.11); small groups and single B-cells (fig. 1.12) have oval or polygonal form contains deposited insulin.

Victoria 4R staining. A compact groups of 10–15 or single oval form B-cells not completely formed islet; insulin content visually is not reduced comparatively with B-cells of adult rats and rabbits (fig. 1.13).

Diethylpseudoisocyanine staining. Small compact groups of B-cells or disseminated group contains a few B-cells not completed formation of islet; number of cells is 4–5 times less in compared with islets of adult rats and rabbits; insulin content visually is not reduced comparatively with B-cells of adult rats and rabbits (fig. 1.14): IF=1,96±0,09 (intact rat B-cells — 2,02±0,08).

Immunofluorescent method. Compact small groups contains 12±7,6 B-cells visually almost completed forming of small islet; insulin content (green fluorescence) visually is not reduced comparatively with B-cells of adult rats (fig. 1.15). On sections prepared using pancreas tissue, around islets are located exocrine tissue.

State of histostructure and insulin content in islets after action of 8PTSQ and Dithizon

Aldehyde-fuchsine staining. Intact islets: oval form, histostructure without changes, a large amount of deposited insulin (violet color) in cytoplasm of B-cells (fig. 1.16) which maximally are concentrated in B-cells located around blood capillaries; AB=2,01±0,05.

Victoria 4R staining. Intact islets: histostructure of islets without changes; a large amount of deposited insulin (blue color) in cytoplasm of B-cells (fig. 1.17); A-cells on periphery of islets (red color) AB=1,68±0,06.

Immunohistochemistry. Intact islets: histostructure and form of islets without changes; a large amount of deposited insulin (brown color) in cytoplasm of B-cells (fig. 1.18) (AB=1,87±0,07);

Diethylpseudoisocyanine staining. Intact islets: histostructure and form of islets without changes; a large amount of deposited insulin (red fluorescence) in cytoplasm of B-cells (fig. 1.19); IF=2,04±0,06.

Fluorescent staining of Zn^{+2}-ions. Intact islets: intensive green fluorescence of Zn^{+2}-ions B-cells (fig. 1.20): IF=2,08±0,05.

Aldehyde-fuchsine staining. Past action of 8PTSQ: destruction and death of majority of B-cells, marked decreasing of insulin content in B-cells (AB=1,12±0,03) (fig. 1.21).

Victoria 4R staining. Past action of DZ: destruction and death of majority of B-cells, marked decreasing of insulin content in B-cells (AB=1,08±0,09) (fig. 1.22); reduced size of islets; A-cells on periphery of islets without changes.

Immunohistochemistry. DZ: decreasing of insulin content in 90–95 % of B-cells (AB=1,03±0,02) (fig. 1.23); reduced size of islets.

Diethylpseudoisocyanine staining, Past action of TSQ: destruction of B-cells in central part of islet; marked decreasing of insulin content in B-cells (IF=1,11±0,04) (fig. 1.24).

Fluorescent staining of Zn^{+2}-ions, DZ: absence of Zn^{+2}-ions in cytoplasm of B-cells (fig. 1.25): IF=2,08±0,05) (fig. 1.25).

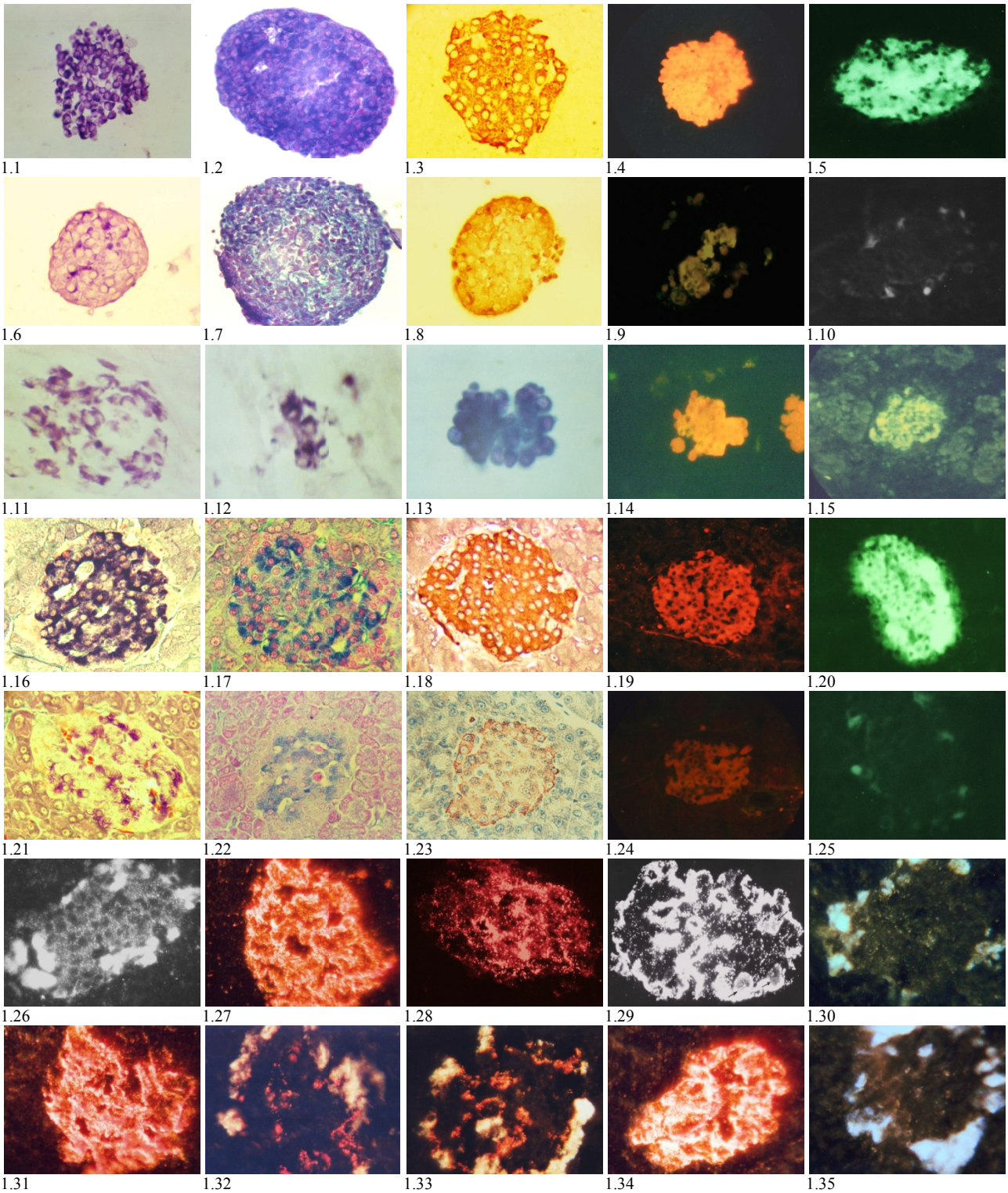

Figure 1

1.1–1.5 Intact isolated pancreatic islets. Histostructure without changes. Normal content of Insulin and Zn^2-ions in B-cells: high density of staining and intensive fluorescence of B-cells; 1.1 — Aldehyde-fuchsine; 1.2 — Victoria 4R; 1.3 — Immunohistochemistry; 1.4 — Diethylpseudoisocyanine; 1.5 — 8PTSQ

1.6–1.10 Pancreatic islets treated by Dithizon. Destruction and death of B-cells; marked decreasing of Insulin and Zn^2-ions in B-cells; 1.6 — Aldehydefuchsine; 1.7 — Victoria 4R; 1.8 — Immunohistochemistry; 1.9 — Diethylpseudoisocyanine; 1.10 — 8PTSQ.

1.11–1.15 Human embryon's small pancreatic islets and not formed groups of B-cells. Almost normal content of Insulin in B-cells; 1.11 — Aldehydefuchsine; 1.12 — Victoria 4R; 1.13 — Immunohistochemistry; 1.14 — Diethylpseudoisocyanine; 1.15 — Immunofluorescent method for Insulin.

1.16–1.20 Pancreatic islets of intact pancreas tissue. Histostructure without changes. Normal content of Insulin and Zn^2-ions in B-cells: high density of staining and intensive fluorescence of B-cells; 1.16 — Aldehyde-fuchsine; 1.17 — Victoria 4R; 1.18 — Immunohistochemistry; 1.19 — Diethylpseudoisocyanine; 1.20 — 8PTSQ.

1.21–1.25 Pancreas of animals with experimental diabetes. Destruction and death of B-cells; marked decreasing of Insulin and Zn^2-ions in B-cells; 1.21 — Aldehyde-fuchsine; 1.22 — Victoria 4R; 1.23 — Immunohistochemistry; 1.24 — Diethylpseudoisocyanine; 1.25 — 8PTSQ.

1.26–1.30 Pancreas of intact animals (1.25), past injection of DZ (1.27–1.29) and past injection of DDCA and DZ (1.30); Staining by Dithizone.

1.31–1.35 Pancreatic islets past injection of DZ (1.31), Cystein + DZ: prevention formation of complex DZ–Zn^2 (1.32), Gluthatione Reduced form + DZ; prevention formation of complex DZ–Zn^2 (1.33), Gluthatione Oxidized form + DZ; not prevention formation of complex DZ–Zn^2 in B-cells (1.34), Histidine + DZ; prevention formation of complex DZ–Zn^2 (1.35); 1.31–1.35 staining by DZ; ×140 for fig. 1.2, 1.10, 1.15, 1.20; ×280 for other preparats.

Influence of Cystein, L-Hystidin and DDCA on interaction of Zn^{+2}-ions of B-cells with Dithizon

Intact pancreas tissue

Investigation of intact frozen sections of rabbit and mice pancreas without staining showed using dark microscopy: histostructure of islet without changes; on periphery lo- cated white color A-cells (fig. 1.26). Injection of Dithizon to animals accompanied a few minutes later by formation of a large amount of red granules of DZ-Zn^{+2} complex in B-cells (fig. 1.27, 1.28).Concentration of complex in islets of mice is low comparatively with rabbits: AB rabbit=2,02±0,04; AB mice=1,62±0,14. This is determined by reduced concentration of Zn^{+2}-ions in B-cells of mice. Investigation of disposition of granules of DZ-Zn^{+2} complex in islets evidently demonstrated that this is maximal concentration of complex around capillaries (fig.1.29). Elimination of Zn^{+2}-ions from B-cells result prevention formation of DZ-Zn^{+2} in islets (fig.1.30) as by prevention developing of diabetes caused by DZ in animals (9,20).

Past action of Cystein, L-Hystidin and DDCA

Obtained results showed that injection of Cystein and L-Hystidine result almost complete binding of Zn^{+2}-ions in cytoplasm of B-cells. Minimal amount of toxic complex as DZ-Zn^{+2} was formed in B-cells contacted with islet arterial capillaries (fig. 1.32,1.33) in compared with a large amount of complex completed all surface of cytoplasm of B-cells not only around capillaries in intact islets (firg. 1.31). Results of measuring of amount of complex in cytoplasm. Intact islets: AB=2,02±0,04; Cystein: 1,21±0,02; L-Hystidine: 1,32±0,02.

Injection of DDCA 500 mg/kg and 1000 mg/kg accompanied by complete binding of Zn^{+2}-ions in cytoplasm of B-cells that result prevention formation of DZ-Zn^{+2} complex (fig. 1.30); AB=1,01±0,02.

Table 1

Comparative analysis of results of measuring of insulin content in B-cells

№	Method	Intact animals	Diabetes	Difference intact/diabetes
	Isolated islets			
1	Aldehyde-fuchsine	1,93±0,06	1,14±0,04	0,79
2	Victoria 4R	1,97±0,05	1,29±0,05•*	0,59
3	Immunohistochemistry	1,81±0,04*	1,18±0,04	0,63
4	Diethylpseudoisocyanine	2,02±0,05*	1,07±0,01•*	0,95
	Pancreas tissue			
1	Aldehyde-fucshine	2,01±0,05•	1,12±0,03	0,89
2	Victoria 4R	1,68±0,06•**	1,08±0,09	0,60
3	Immunohistochemistry	1,95±0,07	1,03±0,02	0,84
4	Diethylpseudoisocyanine	2,04±0,06**	1,11±0,04	0,93

Note. $P < 0,001$.

A comparative analysis of results using of different histochemical methods staining of insulin showed that most precise results of quantitative estimation of insulin content in B-cells were obtained using Immunohistochemical and Diethylpseudoisocyanine methods. We explain it by high specifity of these methods for insulin; there is no staining of other substances or structures in B-cells using these methods that is

why they does not can to change results of photometry. Analysis showed also the presence of higher values of insulin content using Diethylpseudoisocyanine method comparatively with immunohistochemical technique that is determined by evidently more high sensitivity of Diethylpseudoisocyanine method as fluorescent method. Minimal value of parameter K as 1,68±0,06 (Table 1)was obtained using of Victoria 4R method that is explained by high level of absorbance by exocrine tissue.

Results of investigation of staining of B-cells of human embryo showed that majo- rity of investigated small islets not completed forming. There are groups consisting of a few B-cells. They were not yet formed islet as organ: a circulatory system capillaries and capsule were absent.

The insulin content in B-cells is almost same as in B-cells of rats. We observed also a multiple single B-cells or very small groups consisting from 2–3 of cells.

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В-цитотоксикалық заттар әсерінен ұйқы безінің эндокринді ұлпа жағдайын гистохимиялық және иммуногистохимиялық зерттеу

Авторлар анықтағандай, көктамырға цистеин мен L-гистидин аминқышқылын енгізгеннен кейін мырыш байланыстырушы диабетогенді заттар тудыратын жануарларда диабеттің 1-түрі дамуын 90 % алдын алады. Барлық жағдайда бұл аминқышқылдарын енгізгеннен кейін, В-жасушаларды бұзатын токсикалық кешен түзетін мырыш байланыстырушы В-жасушалар диабетогенді заттар толық жойылуымен байқалды. Авторлар цистеин және L-гистидин алдын алу әрекетін, мырыш иондарымен токсикалық емес кешен түзілуі SH-топ молекулаларының дитизон кешенін қалыптасуын болдырмауын жорамалдайды. Авторлар көрсеткендей, В-жасушаларда инсулинді гистохимиялық және иммуноцитогистохимиялық әдістерден басқа, флюоресценттік әдістер, ал Виктория 4R және альдегидфуксинді әдістер панкреатит аралшаларының гистоқұрылымдық күйін бағалауға мүмкіндік береді.

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Гистохимическое и иммуноцитогистохимическое исследование состояния эндокринной ткани поджелудочной железы после воздействия В-цитотоксических веществ

Авторами установлено, что внутривенное введение аминокислоты цистеина и L-гистидина животным в 90 % случаев предотвращает развитие диабета 1 типа, вызываемого цинксвязывающими диабетогенными веществами. Показано, что во всех случаях введение этих аминокислот сопровождается почти полным отсутствием связывания цинка В-клеток с диабетогенными веществами с образованием токсичных комплексов, разрушающих В-клетки. Авторы предполагают, что предупреждающее действие цистеина и L-гистидина обусловлено содержанием в молекуле SH-групп, через которые формируется нетоксичный комплекс с ионами цинка, препятствующий формированию комплексов с дитизоном. Авторами также показано, что из использованных гистохимических и иммуноцитогистохимических методов количественной оценки содержания инсулина в В-клетках наиболее точные результаты обеспечивают иммуногистохимический и диэтилпсевдоизоцианиновый методы, а альдегидфуксиновый метод и метод Виктория 4R наиболее подходят для оценки состояния гистоструктуры панкреатических островков.

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