UDC 616-018:616:379-008.64:577:112.386.2

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Histochemical Methods for Identification of Zinc ions in Pancreatic Islets, Prostate and in Salivary Glands

Authors have used two histochemical methods: 8PTSQ, a high specific fluorescent and Dithizon method for identification of Zinc in the frozen and paraffin sections of tissues of pancreas as in tissues of prostate and salivary glands. It was showed that 10–15 min past one injection to animals of diabetogenic chelators (DC) result accompanied by binding of Zn ions and by negative reaction for Zn in B-cells determined absence of free Zn ions for staining. In the contrary, 5–7 days past injection of DC reaction for Zn in B-cells was negative as result of destruction of cells and of almost complete disappearing of Zn ions from B-cells. Analogical negative reaction for Zn ions there are in B-cells past elimination of Zn by Glibenclamide. Meanwhile administration of Glibenclamide accompanied by elimination of Zinc ions from B-cells only, not from cells of prostate and from salivary glands. It was demonstrated that Dithizon method is more preferable for more detail investigation of location of Zinc in various parts of B-cells. The advantage of 8PTSQ fluorescent method determined by more high sensitivity in compared with Dithizon method. It is established that lifetime coloring of pancreatic B-cells of islets, of trailer part of epithelium cells of Prostate and salivary glands after administration of Ditizon to animals allows to study in detail in them location of Zinc using microscopy in the dark field.

Key words: pancreatic islets, prostate gland, salivary glands, zinc, dithizon, chelates.

Important role of Zinc in organism of animals and human determined by high biological activity. Biological significance of Zinc is determined as by component of some enzymes. Zinc take part in metabolism of nucleic acids and in processes of synthesis of proteins [1, 2].

Zinc takes part also in metabolism and realization effect of hormones of hypophysis, adrenal glands, pancreas, prostate and testicles [3]. The hypophysis, pancreas, eye retina, and prostate contains a large amount of Zinc [4].

A large amount of Zinc ions is revealed in pancreas and its prevalence in pancreastic islets is [5] determined by biosynthesis of insulin in B-cells. It is known that insulin is synthesized and stored in B-cells as insulin deposited crystal form Zinc-insulin complex in the ratio 2:6. Is supposed that releasing of crystal insulin from B-cells accompanied by dissolving of crystals and hexamer dissociate with forming of active monomers of insulin and ions of Zn^{+2} [4, 5].

The tissue of prostate gland contain more than 10 times high amount of Zinc comparatively with other tissues. A large amount of Zinc in prostate protect it, as supposed, of inflammation and improve local immunity against infections [6–8]. Zinc is need for normal function of organs of taste. He stimulates synthesis of a gustin — a protein contain histidine, a component of product of salivary glands. Pancreas, prostate and salivary glands cells carry not only accumulation of Zinc but of its secretion [9].

There are a various methods measuring of concentration of Zinc in biological liquids and in tissues. The following methods are the most widely used today: ardent nuclear and absorbing spectrometry, nuclear and

issue spectrometry with inductive connected plasma, the neutron-activation analysis, X-Ray-spectrometry, an anode inversion volt-amperometry [10, 11]. Along with their indisputable advantage for revealing of very small amount of chemicals in various objects, they are not suitable for to estimate dynamics of content of metals in cells and tissues of bodies in vivo and in vitro and also for identification of cytological bases mechanisms of development of pathological states caused by their deficiency.

Our attention was attention was drawn by methods of research which possess a high sensitivity for identification of Zinc and possibility to observe cells visually. Ditizon (diphenylthiocarbazon, DZ) is widely used in analytical chemistry for detection of heavy metals including Zinc with which he forms chelat complexes. It was showed by using of spectral analysis that maximum of absorbance of complex Zn^{+2}-DZ extracted from pancreatic islets of pancreas tissue is equal to 530 nm that correspond to maximum of absorbance of artificial complex Zn^{+2}-DZ formed in vitro as result of interaction of Zn ions with DZ [12–14].

E.A. Bozhevolnov reported about ability of 8 aren(sulphonylamino)quinolines to form complexes fluorescing under ultra-violet light with Zinc and Cadmium. One of these derivatives — a 8-para(toluenelsulphonilamino)quinoline (8PTSQ) — possess ability to form chelat complexes with Zn^{+2} ions as 1:1. This method of histochemical identification of Zn ions is high specific and very sensitive, allowing to reveal very low concentrations of Zn ions correspond to 10^{-7}–10^{-8} [15]. In ultra-violet light — wavelength equal of 360–370 nm — the Zn-8PTSQ complex fluoresces as brightly green light complex [8]. These chelat active chemicals possesses high chemical affinity to Zn ions and in the conditions of in vitro formed color chelat complexes Zn-8PTSQ visible at luminescent microscopy and a complex as Zn-Dithizon (DZ) visible as bright red granules using microscopy in the dark field [13, 14, 16]. One this base we suppose that these methods could be suitable for histochemical identi fication of Zinc in various bodies and tissues differing in his contents both at a physiological state and when modeling any pathology.

Research objective: to reveal of Zinc ions in pancreatic islets, prostate and in salivary glands of mammals by using of high specific histochemical Dithizon and 8PTSQ methods.

Research Methods

For experiences 26 rabbits males, 2450–2850 g, and 16 white mice 30–36 g were used. Animals have been distributed for 4 groups. Group 1: rabbits, mice; vital staining of tissues by intravenous injection of Dithizon and 8PTSQ. 10 min later past injection tissues of pancreas, prostate and salivary glands were frozen in cryostat. Sections of tissues 5 mcm were investigated in fluorescent microscope and using of dark microscopy.

Group 2: two models of experimental diabetes were induced in animals: 1) by intravenous injection to rabbits and mice of water-ammonia solution of a Dithizon («SERVA», Germany) 45–51 mg/kg and to rabbits — of ethanol solution of 8PTSQ (Institute for Pure Reagents, IREA, Moscow, Russia), 36–38 mg/kg.

Group 3. Peroral administration to animals of the Glibenclamide («Berlin-Chemie», Germany), 20–25 mg/kg daily during 3 days for maximal elimination of Zinc ions from cells.

Group 4. Control intact animals. Administration of equivalent volumes of physiological solution. Fixation of tissues of pancreas, prostate and salivary glands of animals of groups 2, 3, 4 at temperature of 0…–5 ºC in 70º etanol saturated with hydrogen sulfide.

For histochemical fluorescent staining of Zn ions 0,04 % acetone solution of 8PTSQ was used: 3–4 drops of solution placed on sections of tissues for 8–10 sec. following washing in the distilled water. Then sections were investigated using fluorescent microscopy [15].

Preparing of Ditizon solution. For preparation of solution of Ditizon: 30 ml of distilled water, 0,6 ml of 25 % of solution of NH_4OH and 400 mg of Ditizon were placed in vessel. Solution was mixed on a water bath (+70 ºC) within 10 min., filtered using of ashless filter. The filtrate contains approximately 1 % water-ammoniac solution of Ditizon which we used in our researches [13, 14].

Universal microscope Axioplan 2 for light and fluorescent microscopy, photometric system and digital millivoltmeter were used for measuring intensity of fluorescence or intensity of luminescence of B-cells on sections of pancreas with registration of intensity past staining of sections by 8PTSQ or by Dithizon respectively. Cytochemical indicators measuring of Zinc ions content were estimated as conventional units (c.u.) [17, 18].

For statistical analysis the Statistica 8,0 (Stat Soft Inc) and Microsoft Excel 2006 were used with calculation of M+m. After checking of distribution to a normality the importance of distinctions between groups was estimated by means t-criterium of Student's. Distinctions were reliable at 95 % a probability threshold ($p < 0,005$).

Results

For the first it was showed that intravenous administration to rabbits and to mice of Ditizon and 8PTSQ 5 min. later result formation of specific complexes Zn-8PTSQ and Zn^{+2}-DZ visible on frozen sections in cells contains a large amount of Zn ions: B-cells of pancreatic islets, cells of tissues of prostate and salivary glands (Fig. 1.1–1.3, 1.7, 1.8, 1.10–1.12). Same result we show in paraffin staining sections of fixed tissue of pancreas. Intensity of staining (Dithizon) and of fluorescence (8PTSQ) of tissues was measured in compared with control. The results showed a positive reaction for Zn as intensive red color of B-granules in B-cells past injection of Dithizon (Table: 1,94±0,05; 2,76±0,08; 2,89±0,08; Fig. 1.7, 1.8) as in cells of Prostate and in Salivary glands. In the contrary, we have observed negative reaction for Zn past staining by 8PTSQ past injection of Dithizon (Table 1: 1,07±0,03; 1,15±0,04; 1,09±0,05) that was determined by preliminary formation of complex Zn-Dithizon. As result, staining of sections by 8PTSQ not accompanied by forming of fluorescent complex Zn-8PTSQ due to absence of free Zn ions in B-cells for interaction with 8PTSQ.

Dithizon and 8PTSQ formed in B-cells chelat complexes with Zn that result destruction of cells and absence of Zn from B-cells (Fig. 1.6.). Taking into consideration this fact we tried to eliminate of Zinc ions from cells by Glibenclamide for try to protect B-cells of formation into cells of chelat complexes and by using of this way try to protect cells of destruction and of developing of diabetes. For realization of this purpose Glibenclamide was entered daily to animals of Group 3 which possess hypoglycemic effect due to ability to stimulate dissociation of Zinc-insulin complex in B-granules of B-cells and releasing of free insulin and Zinc ions in the blood. Results showed marked decreasing of content of Zinc ions in B-cells for 1,9–2 times in sections of pancreas tissue past staining by 8PTSQ and by Dithizon respectively ($p < 0,001$).

Elimination of Zn from B-cells by Glibenclamide accompanied by negative reaction for Zn using of both methods (Table; Fig. 1.4, 1.5, 1.9). In the contrary, using of Glibenclamide not accompanied by elimination of Zn from cells of Prostate and of Salivary glands (Table). Results showed that intensity of fluorescence of complex Zn-8PTSQ as intensity of color of complex Zn-Dithizon was approximately for 1,5–1,6 times more high comparatively with B-cells.

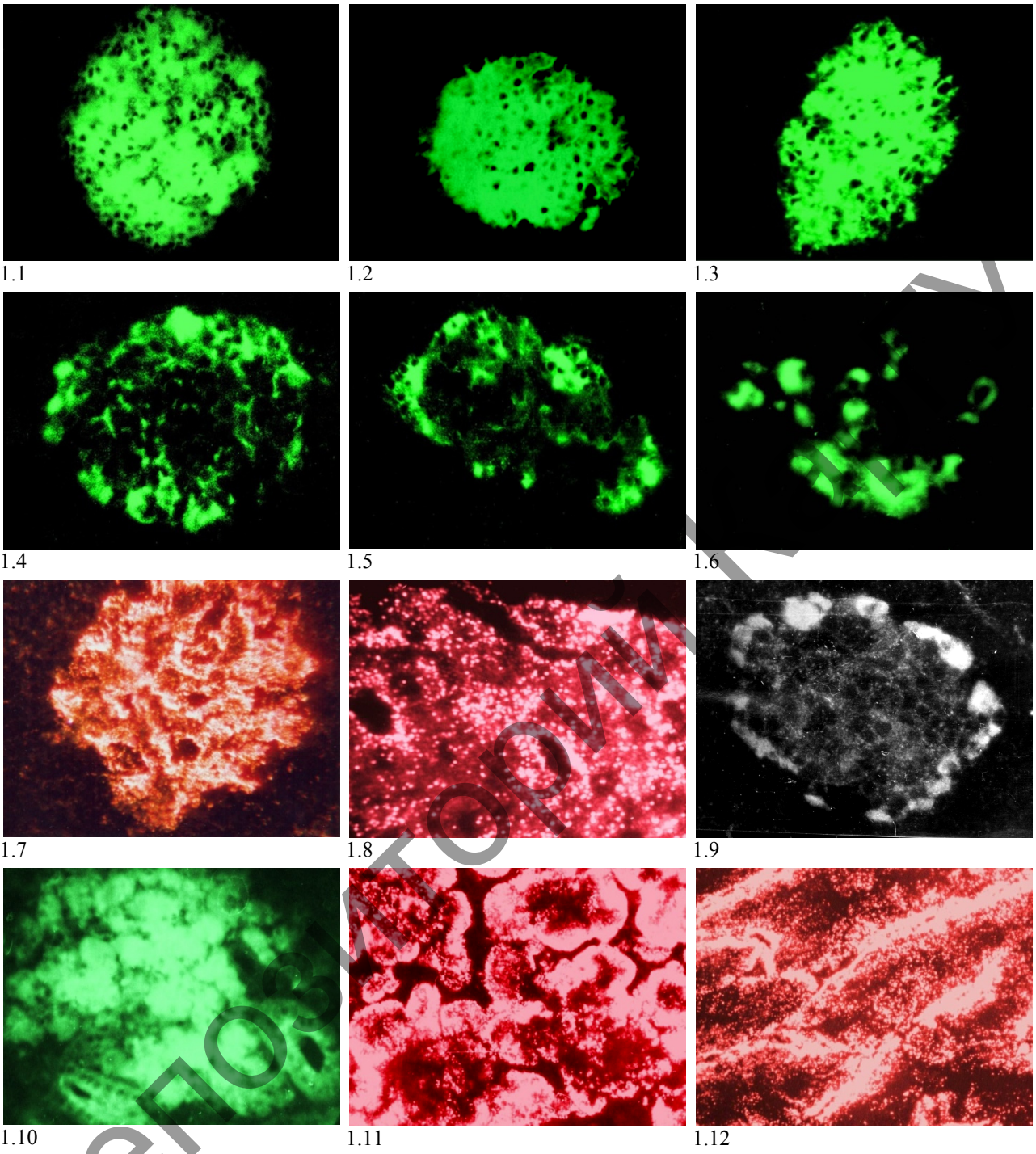
T a b l e

Histochemical methods for revealing of Zinc ions in Pancreas, Prostate and Salivary glands (M±m)

Groups of animals	Conditions of experience	Zinc ions content in tissues (c.u.)					
		Intensity of fluorescence			Intensity of staining		
		Staining of Zn by 8PTSQ			Staining of Zn by DZ		
		B-cells of pancreas	Prostate	Salivary gland	B-cells of pancreas	Prostate	Salivary gland
1	Dithizon, 50,2 mg/kg	1,07±0,03* *n*=16	1,15±0,04^{+} *n*=16	1,09±0,05** *n*=16	1,94±0,05 *n*=16	2,76±0,08 *n*=16	2,89±0,08 *n*=14
2	Experimental diabetes	1,04±0,05* *n*=14	3,04±0,12 *n*=14	3,32±0,11 *n*=18	1,03±0,07*** *n*=14	2,05±0,09 *n*=14	2,20±0,14 *n*=14
3	Glibenclamide, 25 mg/kg	1,05±0,04* *n*=19	2,95±0,11 *n*=19	3,40±0,20 *n*=19	1,06±0,08*** *n*=19	2,08±0,11 *n*=19	2,28±0,08 *n*=19
4	Intact animals (control)	2,07±0,08 *n*=14 *p*<0,005	3,12±0,09 *n*=21 *p*<0,001	3,35±0,12 *n*=16 *p*<0,001	1,92±0,06 *n*=12 *p*<0,001	2,95±0,09 *n*=15	3,05±0,06 *n*=12

It was especially interesting fact that we did not observe same changes of the Zinc ions content in cells prostate and salivary glands in which intensity of a fluorescence and luminescence significantly did not differ from analogical indicators in control sections. We have not found analogical changes of Zinc content in tissues of prostate and salivary glands Indicators of intensity of fluorescence and of luminescence significantly didn't differ from indicators in control (Table).

This fact demonstrates a selective influence of Glibenclamide on Zinc-insulin complex which is localized as depot form in the B-granules of pancreatic β-cells. Meanwhile, administration to animals as of diabetogenic chelat active chemicals as of Glibenclamide accompanied by negative reaction for insulin in B-cell only, not in A-cells. On the base of obtained results it is possible to suppose the presence of various conditions of metabolism of Zinc ions: regulation of metabolism by metalthioneins [10, 19, 20] of transport by transmembrane proteins[7, 21–23], deposition and of excretion [8, 24, 25].



1.1 1.2 1.3

1.4 1.5 1.6

1.7 1.8 1.9

1.10 1.11 1.12

1.1 Frozen section of pancreas tissue of Rabbit 10 min. past injection of 8PTSQ. Fluorescent reaction with 8PTSQ. Green fluorescence of Zn-8PTSQ complex; ×140;

1.2 Pancreatic islet of intact Rabbit. Histochemical revealing of Zinc ions by 8PTSQ; ×140;

1.3 Pancreatic islet of intact Mice. Histochemical revealing of Zinc ions by 8PTSQ; ×140;

1.4 Pancreatic islet of Rabbit 3 days past administration of Glibenclamide. Elimination of Zincions from B-cells; negative reaction or Zn in B-cells of central part of islet; staining by 8PTSQ.; ×140;

1.5 Pancreatic islet of Mice 3 days past administration of Glibenclamide. Elimination of Zinc ions from B-cells; negative reaction for Zn in B-cells of central part of islet; staining by 8PTSQ.; ×140;

1.6 Pancreatic islet of Rabbit 14 days past administration of Dithizon. Absence of Zinc ions in B-cells; staining by 8PTSQ; ×140;

1.7 Pancreatic islet of Rabbit 10 min. past administration of Dithizon. Red granules of complex Zn-DZ in B-cells. Dark microscopy; ×280;

1.8 Pancreatic islet of Mice 10 min. past administration of Dithizon. Red granules of complex Zn-DZ in B-cells. Dark microscopy; ×800;

1.9 Pancreatic islet of Rabbit Administration of Glibenclamide within 7 days + injection of Dithizon; absence of complex Zn-Dz in B-cells; dark microscopy; ×280.

1.10 Frozen section of salivary gland of rabbit past intravenous administration of 8PTSQ. Intensive green fluorescence of complex Zn-8PTSQ; fluorescent microscopy; ×140;

1.11 Frozen section of prostate of rabbit past intravenous administration of Dithizon. Red granules of complex Zn-Dithizon; dark microscopy; ×280;

1.12 Frozen section of salivary gland of rabbit past intravenous administration of Dithizon. Red granules of complex Zn-Diithizon; dark microscopy; ×280№

Figure 1. Histochemical methods staining of Zinc in cells

The histochemical method revealing of Zinc using of 8-para(toluenesulphonylamino)quinolin is a most high sensitive for Zinc ions, which forming with him the specific chelat complex fluorescing in ultraviolet light [13, 15]. The advantage of Dithizon method of histochemical identification of Zn ions based on ability to form the complexes having not diffusion coloring but brightly red granules of complex Zinc-DZ in the ratio 2:1 that allows to study metal cytotopography considering various concentration of localization granules in various parts of cells. Our results don't contradict data of other authors who also studied at the cellular level dynamics of Zinc content at animals under various experimental conditions [12, 14].

Thus, results of the comparative analysis of two histochemical methods staining of Zinc ions in cells of various bodies allow to confirm their high sensitivity, and possibility to use for cytologic researches.

Acknowledgement

Gratitude to Prof. G.G. Meyramov and Prof. V.I. Korchin, Russia for staining technologies and financing of investigation.

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Ұйқы, қуық асты және сілекей бездерінде мырышты анықтаудың гистохимиялық әдістері

Тіндердің үлгілерін алу және кәдімгі гистологиялық әдістер арқылы ағза тіндердегі микроэлементтердің иондарын анықтауда гистохимиялық әдістер әрқашан оң нәтиже бере алмайтыны белгілі. Ұйқы безінің тіндеріндегі металдарды анықтаудағы люминесцентті әдістері үшін кәдімгі бекіту әдістері іс жүзінде жарамсыз болып табылады. Авторлар қуық асты, сілекей және ұйқы бездерінің В-жасушалардың кесілген бөліктерінің жаңа мұздатылған бөліктерінде мырышты анықтауда жоғары нақты дитизиондық және люминесцентті 8TCX гистохимиялық әдістер пайдаланған. Сусамыр (қант диабетінің) хелаттүзуші қуық асты және сілекей бездерінің В-жасушаларында мырыш теріс реакцияға әкелді: ол мырыш қатысуымен еркін мырыш теріс реакция жоқтығын растаған, осы әдістер В-жасушалардың мырыш бұғаттау эксперименттері ретінде көрсетілген. Кіріспе глибенкламид енгізгенде В-жасушаларынан мырышты шығарумен қатар, мырыш теріс реакциясының пайда болуы байқалды. Ұйқы безінің және В-жасушаларындағы мырыш иондарының орналасуы дитизиондық әдістің көмегімен зерделенді, ал 8TSKH әдісі жоғары сезімталдық артықшылығымен ерекшеленді.

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Гистохимические методы выявления цинка в панкреатических островках, предстательной и слюнных железах

Известно, что выявление ионов микроэлементов гистохимическими методами в тканях организма далеко не всегда дает положительный результат при использовании обычных гистологических методов фиксации и проводки образцов ткани. А для люминесцентных методов выявления металлов в ткани поджелудочной железы обычные методы фиксации вообще практически непригодны. Авторами использованы высокоспецифичные дитизоновый и люминесцентный 8TCX гистохимические методы выявления цинка в срезах не фиксированной, а свежезамороженной ткани предстательной и слюнных желез и в В-клетках поджелудочной железы. Результаты свидетельствуют о том, что в панкреатических островках животных с помощью обоих методов выявлена резко положительная реакция на цинк во всех исследованных тканях. Показано, что в опытах с блокированием цинка в В-клетках данные

методы подтвердили отсутствие свободного цинка наличием отрицательной реакции на цинк: однократное введение диабетогенных хелаторов приводило к отрицательной реакции на цинк в В-клетках, в предстательной и слюнных железах. Введение глибенкламида, сопровождающееся выведением цинка из В-клеток, приводит к появлению также отрицательной реакции на цинк. Показано, что дитизоновый метод позволяет детально изучить расположение ионов цинка в панкреатических островках и в В-клетках. 8ТСХ метод имеет преимущество в виде более высокой чувствительности.

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