UDC 616.379-008.64-092.9

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Histochemical method for fluorescent staining of Zn^{+2}-ions in glands

It is showed by authors that using of 8-para(toluenesulphonylamino)quinoline — a derivative of 8-oxyquinolin result histochemical revealing by using of fluorescent microscopy of Zn^{+2}-ions in cells of tissue of prostate, in pancreatic B-cells and in salivary glands contains a large amounts of ions. This method is high sensitive and high specific for revealing of Zn^{+2}-ions and there are only one step of staining procedures. Meanwhile it is possible to use only fresh frozen sections of tissues for investigation within short time limited by 15–20 min.

Key words: B-cells, Prostate, Salivary glands, 8-para(toluenesulphonylamino)quinolone, Zn^{+2}-ions.

Background. Pancreatic B-cells contains a large amount of Zn^{+2}-ions [1–3] as salivary glands and prostate. In B-cells Zn^{+2}-ions take part in processes of biosynthesis of insulin as in storage by forming of zinc-insulin complex [4, 5]. It is known that Zn^{+2}-ions in B-cells formed with insulin a deposited form of hormone as Zn^{+2}-insulin complex [4]. Proinsulin forms a Zn^{+2}-ions containing hexamer soon after its synthesis. In addition the zinc ions enhance proinsulin solubility and render insulin insoluble. Pancreas of animals as of Human contain Zn^{+2}-ions [6].

There are between insulin and zinc content in B-cells: decreasing of insulin con- tent accompanied by decreasing of amount of Zn^{+2}-ions and in opposite in intact B-cells a large amount of insulin accompanied by a large amount of Zn^{+2}-ions. Meanwhile for estimate ability of B-cells for storage of insulin in cells it is necessary to use method of staining of zinc-ions.

Some diabetogenic derivatives of 8-oxyquinolin [8OXQ] possess high chemical affinity for Zn^{+2}-ions and in vitro formed color complexes as Zn^{+2}-chelator [7]. One of tits, a 8-para(toluenesulphonylamino)-quinoline [TSQ] is used for color revealing of Zn^{+2}-ions in solutions [8].

Aim of work: 1) to investigate Zn^{+2}-ions content in B-cells using staining by TSQ in pancreas tissue, in prostate and salivary glands of intact animals.

*Methods***.** Animals: 8 Rabbits 2,2–2,6 kg. Frozen sections of Pancreas tissue as of Prostate and Salivary glands were used. Group 1: A) Staining of Zn^{+2}-ions in B-cells on sections of intact animal's pancreas tissue, prostate and of salivary gland using of 0,4 % acetone solution of TSQ. B) Injection of TSO, 38,6 mg/kg and fluorescent microscopy of frozen section of pancreas.

Staining procedures

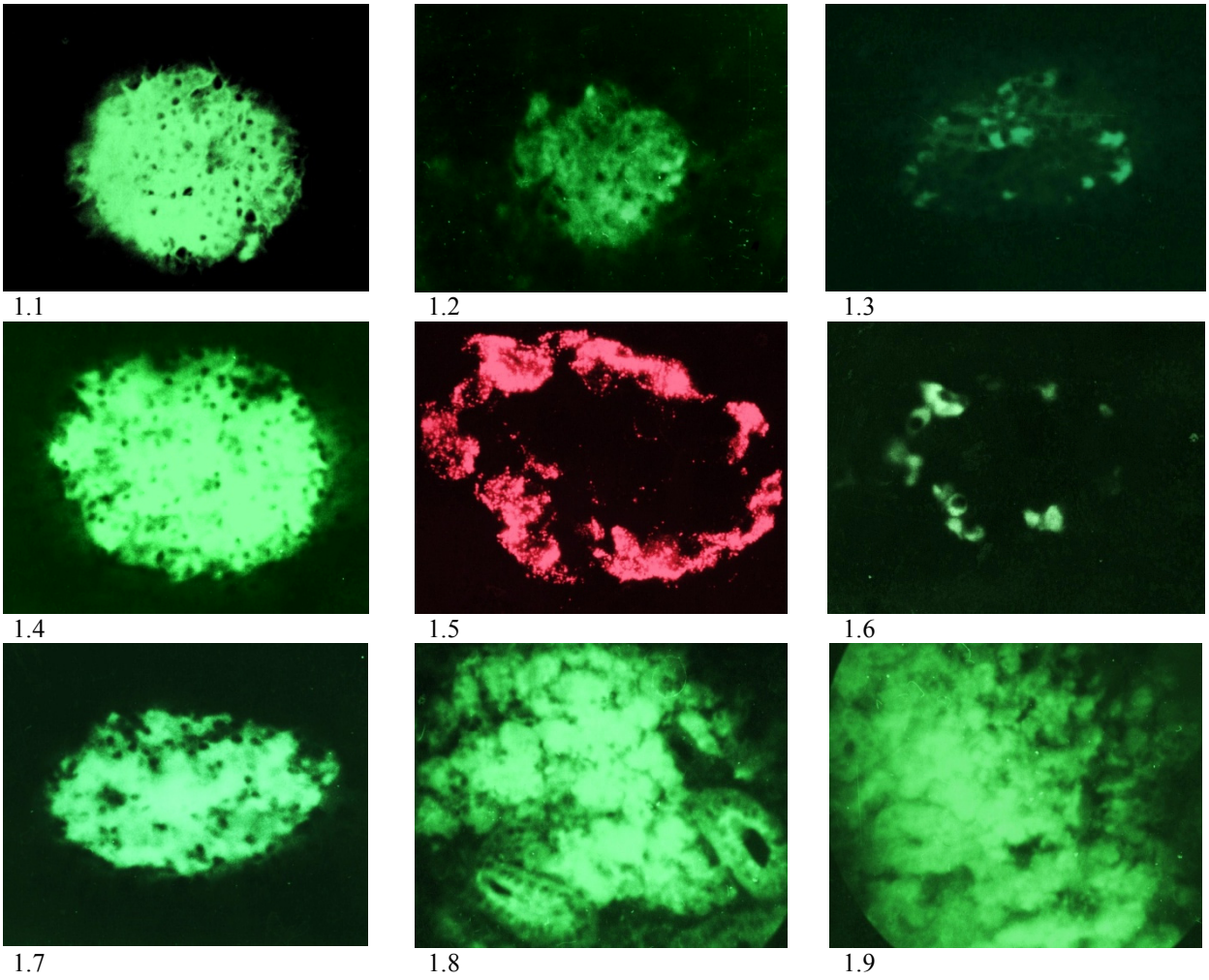
1. Staining procedures for sections of pancreas using fluorescent reagent 8PTSQ: 0,4 % acetone solution of TSQ prepared using NH_4OH 25 %-solution. Staining procedures: a few drops of 8PTSQ solution place on frozen sections for 10 sec.; 3 times washing by distilled water and investigation on UV-light microscope with measuring of intensity of fluorescence (control intensity of fluorescence of exocrine tissue's cells was accepted for 1,00); length of wave of UV-light 360–370 nanometers. For quantitative estimation of results of measuring intensity of fluorescence parameter K was calculated as rate: intensity of fluorescence of B-cells IF1/intensity of fluorescence of exocrine tissue cells IF2 (IF1/IF2);

2. Preparing of TSQ solution for injection (vital staining of Zn^{+2}-ions in islets, prostate and salivary glands): 25 mg of 8PTSQ (Institiute for High Pure Chemicals, Moscow) was dissolved in 70 % Ethanol at $+70^0$ Celsius and injected to Rabbits 36,5–38,8 mg/kg.

3. Staining of insulin and Zn^{+2}-ions content in B-cells of animals with experimental diabetes caused by injection of Dithizone.

Results

1. Intact animals. Intensive fluorescence of complex Zn^{+2}-ions-TSQ as positive reaction for Zn^{+2}-ions in cytoplasm of B-cells was revealed in cytoplasm of B-cells of pancreas past staining by TSQ solution as by using of vital histochemical reaction past intravenous injection of TSQ to animals. We observed decreasing intensity of fluorescence past partial and almost complete removing of Zn^{+2}-ions off B-cells by 3 days prolonged per oral treatment by Glibenclamide, 15–20 mg/kg daily [Fig. 1.2, 1.3].Results of fluorescent microscopy of sections demonstrate partial or almost complete negative reaction for Zn^{+2}-ions — as result of removing of Zn^{+2}-ions from B-cells [Fig. 1.3]. Negative fluorescent reaction for Zn^{+2}-ions in islets of animals with diabetes caused by selective destruction of B-cells by Dithizone (Fig. 1.5, 1.6; Tables 1, 2).

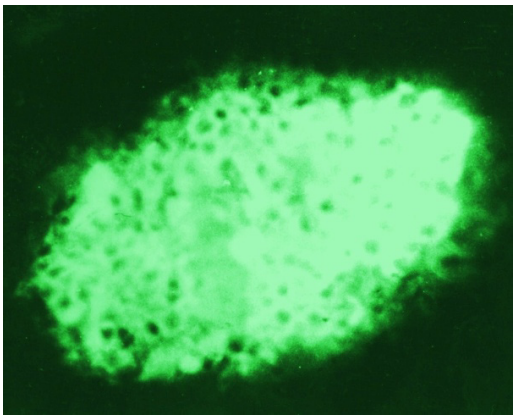


1.1 Intact Rabbit. Fluorescence of Zn^{+2}-ions in B-cells. Frozen section of Pancreas. Staining by 8PTSQ; ×140;
1.2 Injection of DDCA 250 mg/kg. Staining by 8PTSQ; partial binding of Zn^{+2}-ions in B-cells by DDCA; ×140;
1.3 Injection of DDCA 1000 mg/kg. Staining by 8PTSQ; almost complete binding of Zn^{+2}-ions in B-cells by DDCA; negative fluorescent reaction for Zn^{+2}-ions in B-cells; ×140;
1.4 Injection of 8PTSQ 38,8 mg/kg; vital staining of Zn^{+2}-ions in B-cells past injection. Frozen section of pancreas; ×140;
1.5 Rabbit, Destruction of B-cells caused by injection of Dithizone, 48,6 mg/kg; dark microscopy; frozen section of pancreas; ×200;
1.6 Rabbit, Destruction of B-cells caused by injection of Dithizone, 48,6 mg/kg; frozen section of pancreas, staining by 8PTSQ: absence of Zn^{+2}-ions in B-cells;
1.7 Intact Mice. Fluorescence of Zn^{+2}-ions in B-cells. Frozen section of Pancreas. Staining by 8PTSQ; ×140;
1.8 Frozen section of Prostate tissue of Rabbit. Staining by 8PTSQ. Fluorescense of Zn^{+2}-ions; ×140;
1.9 Frozen section of Salivary gland; Staining by 8PTSQ. Fluorescence of Zn^{+2}-ions; ×140

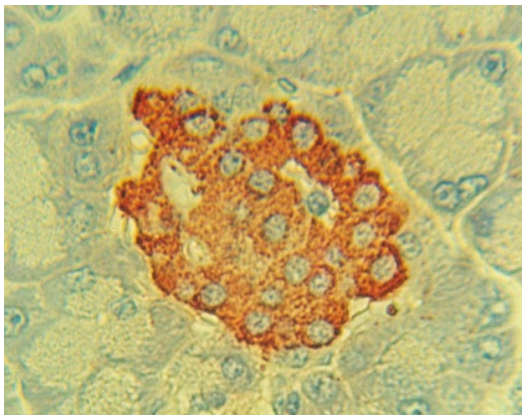
Figure 1

We observed intensive fluorescence of cytoplasm contained a large amount Zn^{+2}-ions cells of Prostate and Salivary gland (Fig. 1.8, 1.9) past intravenous injection of 8PTSQ solution.

2. Animals with diabetes caused by injection of DZ (50,2 mg/kg). Negative reaction for Zn^{+2}-ions with 8PTSQ as for insulin in B-cells in sections of pancreas tissue (fig. 2.3, 2.4; control 2.1, 2.2; Table 1, 2) that demonstrate absence in cytoplasm of B-cells as of Zn^{+2}-ions as of insulin in result of necrosis and destruction of cells: Rabbits: K(IF1/IF2)=1,03±0,05; control: intact B-cells: K=2,06±0,07 ($p<0,001$); Mice: 1,89 ±0,06 and control (intact)=1,06±0,04.Insulin content in B-cells: K(IG1/IG2)=1,12 ±0,03; intact B-cells IG1/IG2=1,92±0,04 (Table 1, 2).



2.1



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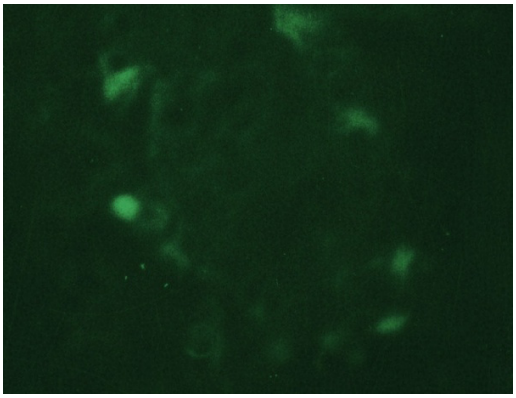

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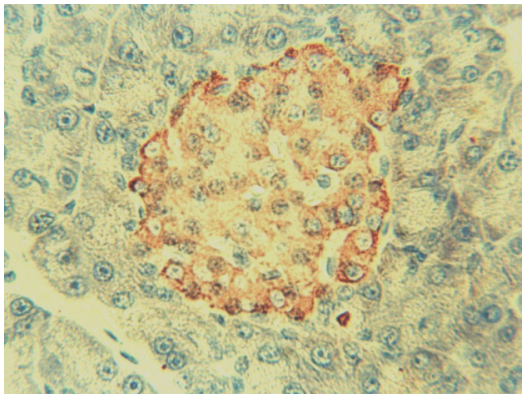

2.4

2.1 Pancreatic islet of intact rabbit. 8PTSQ fluorescent reaction for zinc. Intensive fluorescence (a large amount of zinc in B-cells); UV-light microscopy; ×140;

2.2 Pancreatic islet of intact rat. Immunohistochemical method. Normal content of deposited insulin in B-cells (blue-violet color); ×280;

2.3 Pancreatic islet of rat with dianetes. 8PTSQ fluorescent reaction for zinc. Negative reaction for zinc (absence of fluorescence) determined by destruction of B-cells and by absence of zinc-ions in cytoplasm; UV-light microscopy; ×140;

2.4 Panc reatic islet of rat with diabetes. Immunohistochemical staining method. Decreasing of insulin content in B-cells and of size and number of islets in sections; ×280

Figure 2. Zinc-ions and insulin content in B-cells of intact and experimental rats

Table 1

Zn^{+2}-ions content in B-cells (parameter K: IF1/IF2)

№	Animals	Intact animals (IF1/IF2)	Diabetes caused by Dithizone (IF1/IF2)
1	Rabbits	2,06±0,07•	1,03±0,05•
2	Rats	1,94±0,05	–
3	Mice	1,89±0,06*	1,06±0,04*

Note. • * — $p<0,005$.

Table 2

Insulin and Zinc content in pancreatic B-cells (parameter K)

№	Conditions of experience	Insulin (IG) and Z+2 content (IF) in B-cells (parameter K)	
		insulin (IG)	zinc (IF)
1	5 min. past injection of DZ	1,88±0,05	1,03±0,05
2	Diabetes caused by DZ (48,8–51,6 mg/kg)	1,12±0,03•	1,08±0,03
3	DDCA (987 mg/kg)	1,85±0,04	1,02±0,04*
5	Rabbit (intact)	1,92±0,04•	1,98±0,06*

Note. • * — p<0,001.

Results showed that in 3 cases method demonstrated a full coincidence of Zn^{+2}-ions content with content of insulin in B-cells: 1) in intact animals; 2) in animals with experimental diabetes; 3) in animals after removing of Zn^{+2}-insulin complex from B-cells by drugs.

This method demands following conditions. For fixation of tissue of pancreas to use the 70º alcohol sated with hydrogen sulfide (H_2S) or to use sections of frozen-pancreas tissue. Filters for UV-microscopy: UV-filter between UV-lamp and microscope and yellow filter for ocular of microscope. 8PTSQ is high specific fluorescent reagent for revealing of minimal concentrations of Zn^{+2}-ions in solutions as 10^{-7}–10^{-8}.

References

1 *Eisebrandt J., Scienz M., Wegel F.* Uber den Zinkgehalt menschicher Pancreasdrussen und uber das Bindungs Vormogen des Insulins fur Zink // Medizinund Chemie. — 1942. — No. 8. — P. 259–296.

2 *Schmidt R., Rautschke R.* Der Zink und Kurfergehalt verschiedener Organe der weissen Ratte: ein Beitrag zur Dithizon-Zink-Reaction in der Histochemie // Acta Histochem. — 1964. — Vol. 19. — P. 1–13.

3 *Galabova R., Petkov P., Kolev J.* Rontgen-Fluoreszenz Untersuchung von Zink, Kupfer und Kobalt in Pancreas einiger Sanger // Acta Histochem. — 1971. — No. 2. — P. 335–342.

4 *Andersson T., Betgreen P., Flatt P.* Subcellular distribution of zinc in islets B-cells fractions // Hormones and Metabolism Res. — 1980. — Vol. 12, No. 1. — P. 275–276.

5 *Emdin S.O., Dodson G.G., Cutfield J.M., Cutfield S.M.* Role of zinc in insulin biosynthesis. Some possible zinc-insulin interactions in the pancreatic B-cell // Diabetologia. — 1980. — Vol. 19, No. 3. — P. 174–82.

6 *Okamoto K.* Experimental production of diabetes // Diabetes mellitus: Theory and Practice. — New-York: McGraw-Hill Book company, 1970. — P. 230–255.

7 *Красавин И.А., Бавельский З.Е., Лазарис Я.А., Дзиомко В.М.* Гистохимические реакции на цинк в островках Лангерганса и диабетогенная активность используемых веществ // Проблемы эндокринологии. — М., 1969. — № 3. — С. 102–105.

8 *Божевольнов Е.А., Серебрякова Г.В.* 8-пара(толуолсульфониламино)хинолин — люминесцентный реагент на цинк и кадмий // Химические реактивы и препараты. — М., 1961. — С. 36–42.

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Zn^{+2} иондарын бездерде флюоресцентті анықтаудың гистохимиялық әдісі

Авторлармен 8-пара(толуолсульфониламино)хинолин — 8-оксихинолиннің туындысын қолдану флюоресценттік микроскопияның көмегімен қуықалды безінің ұлпа жасушасында, панкреатикалық В-жасушасында және сілекей бездерінде Zn^{+2} иондарын айқындауға мүмкіндік беретіндігі көрсетілген. Әдістің өзгешілігі — оның жоғары сезімталдығы, жасушаларда Zn^{+2} иондарын айқындау ерекшелігі және боялу тәртібінің бірсатылығы. Салыстырмалы түрдегі кемшілігі — тұрақты гистологиялық препараттарды алу мүмкіндігінің болмауы. Препараттар аз уақыт сақталады — 15–20 мин.

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Гистохимический метод флюоресцентного выявления ионов Zn^{+2} в железах

Авторами показано, что использование 8-пара(толуолсульфониламино)хинолина — производного 8-оксихинолина позволяет с помощью флюоресцентной микроскопии выявлять ионы Zn^{+2} в клетках ткани предстательной железы, в панкреатических В-клетках и в слюнных железах, где он содержится в значительных количествах. Отличительная особенность метода — его высокая чувствительность, абсолютная специфичность в отношении выявления ионов Zn^{+2} в клетках и одноэтапность процедуры окраски. Относительный недостаток состоит в отсутствии возможности получения постоянных гистологических препаратов. Препараты сохраняются относительно недолго — в течение 15–20 мин.

References

1 Eisebrandt J., Scienz M., Wegel F. *Medizinund Chemie*, 1942, 8, p. 259–296.
2 Schmidt R., Rautschke R. *Acta Histochem*., 1964, 19, p. 1–13.
3 Galabova R., Petkov P., Kolev J. *Acta Histochem*., 1971, 2, p. 335–342.
4 Andersson T., Betgreen P., Flatt P. *Hormones and Metabolism Res.*, 1980, 12, 1, p. 275–276.
5 Emdin S.O., Dodson G.G., Cutfield J.M., Cutfield S.M. *Diabetologia*, 1980, 19, 3, p. 174–82.
6 Okamoto K. *Diabetes mellitus: Theory and Practice*, New York: McGraw-Hill Book company, 1970. — p. 230–255.
7 Krasavin I.A., Bavelsky S.E., Lazaris Y.A., Dziomko V.M. *Problems of Endocrinology*, Moscow, 1969, 3, p. 102–105.
8 Bogevolnov E.A., Serebrakova G.V. *Chemicals and drugs*, Moscow, 1961, P. 36–42.

Acknowlegement

Gratitude to Prof. G.G.Meyramov for financing of investigation.