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Victoria-4 histochemical method staining of insulin

Results using of histochemical Victoria-4 method staining of insulin in pancreatic B-cells are presented by authors comparatively with results of using other histochemical methods: immunofluorescent, immunohistochemical, pseudoisocyanine and aldehyde fuchshine. By authors it is shown that this method being specific concerning insulin revealing in B-cells, is not high precise for quantitative measuring of insulin content in cells because not only B-cells but blood vessels and connecting tissue of islets are painted in dark colour registered by a photometer as well as blue colour of B-cells. Using of Victoria-4 method is possible for stain of various histostructures of islets that gives possibility to estimate the state of histostructure of islets not only insulin content.

Key words: insulin contents, immunohistochemical, immunofluorescent, pseudoisocyanine and aldehyde fuchshine methods, B-cells.

There are a few histochemical and immunohistochemical methods staining of insulin in pancreatic B-cells: immunofluorescent (IF), immunohistochemical (IG), aldehydefuchshine (AF) [1], Victoria-4 methods [2–8] and diethylpseudoisocyanine (PS) [7, 9].

Advantage of fluorescent IF and PS methods determined by more high sensitivity of fluorescent stainings: minimal concentration of substances as 10^{-7} – 10^{-8} maybe revealed using these methods. Meanwhile histological sections past staining maybe used for microscopy within short time as 0,5–1 h. Using fluorescent methods not possible to estimate state of histostructure of pancreatic islets. Immunohistochemical method as IF is more high specific for staining of insulin comparatively with all other methods and now widely are used in the world.

Aim of work: to compare results of insulin staining in sections of rat's and rabbit's pancreas tissue using staining by Victoria-4 method [V4] comparatively with immunohistochemical [IG], aldehydefuchshine [AF] and pseudoisocyanine [PS] technologies.

Materials and methods

Pancreas tissue of 12 rats Vistar and 2 rabbits were used. Fixation in Bouin 24 h. Paraffin sections 4 mm were used. Staining methods for insulin revealing in B-cells: Victoria-4 [V4], immunohistochemical [IG], aldehydefuchshine [AF], pseudoisocyanine [PS] and staining by Dithizon [DZ].

The reagent Victoria blue 4R, diphenylnaphthylmethane derivative, mol. wt. 520 ($C_{34}H_{34}N_3Cl$), color Index 42563, «MERCK» (Germany) was used for staining of insulin in B-cells [3]. We have used fixation of pancreas tissue in Bouin, permanganate oxidation of sections prior staining using V4 as a 0.05 % acid alcoholic solution [4, 5].

Mixture for oxidation: 0,3 % aqueous potassium permanganate — 50 ml, 0,3 % Sulphuric acid — 50 ml. Victoria Blue 4R main solution: 96° alcohol — 100 ml, Victoria Blue 4R — 1 g. Victoria Blue 4R staining solution: Victoria Blue 4R main solution — 25 ml, 96° alcohol — 100 ml, Glycerin — 300 ml, 1 % acetic acid glacial — 25 ml [2].

Maximum light absorbtion of V4 solutions depended on the concentration of the reagent. It is suggested that V4 forms molecular aggregates with maximum of absorbtion at 593 nm which could represent monometric dye particles and minimum absorbtion at 540 nm dimeric dye molecules [6]. The nature of the fixation in Bouin on staining of B-cells is not clear. Perhaps this is dependent on the formation of waterinsoluble insulin picrate [4]. Without Bouin fixation crystalline bovine insulin stains by V4 only after oxidation [4, 5].

Insulin content in B-cells was estimated by measuring of absorbance by photometry of B-cells located in central part of islets. Parameter K was calculated. For Pseudoisocyanine and Immunofluorescent methods:

$K=IN1/IN2$; IN1-intensity of fluorescence of B-cells, IN2-intensity of fluorescence of exocrine tissue. For calculation direct dependence was used: increasing of amount of insulin in cells accompanied by higher intensity of fluorescence. For Immunohistochemical, Aldehydefucshine and Victoria-4 methods: $K2=AB1/AB2$; AB1-absorbance of light by exocrine tissue, AB2-absorbance of light by B-cells. For calculation inverse relationship is used: more intensive staining of cells for insulin result reduction of light amount accepted by photometer. By each method 20–25 pairs measurements endocrine/exocrine tissue was used. The average values of K1 and K2 parameters for exocrine tissue was accepted for 1.00.

Results

Insulin in pancreatic B-cells was identified using of all histochemical methods (Table 1, Fig. 1).

Table 1

Intensity staining of B-cells and of exocrine tissue using various methods and insulin content (parameter K) in pancreatic B-cells

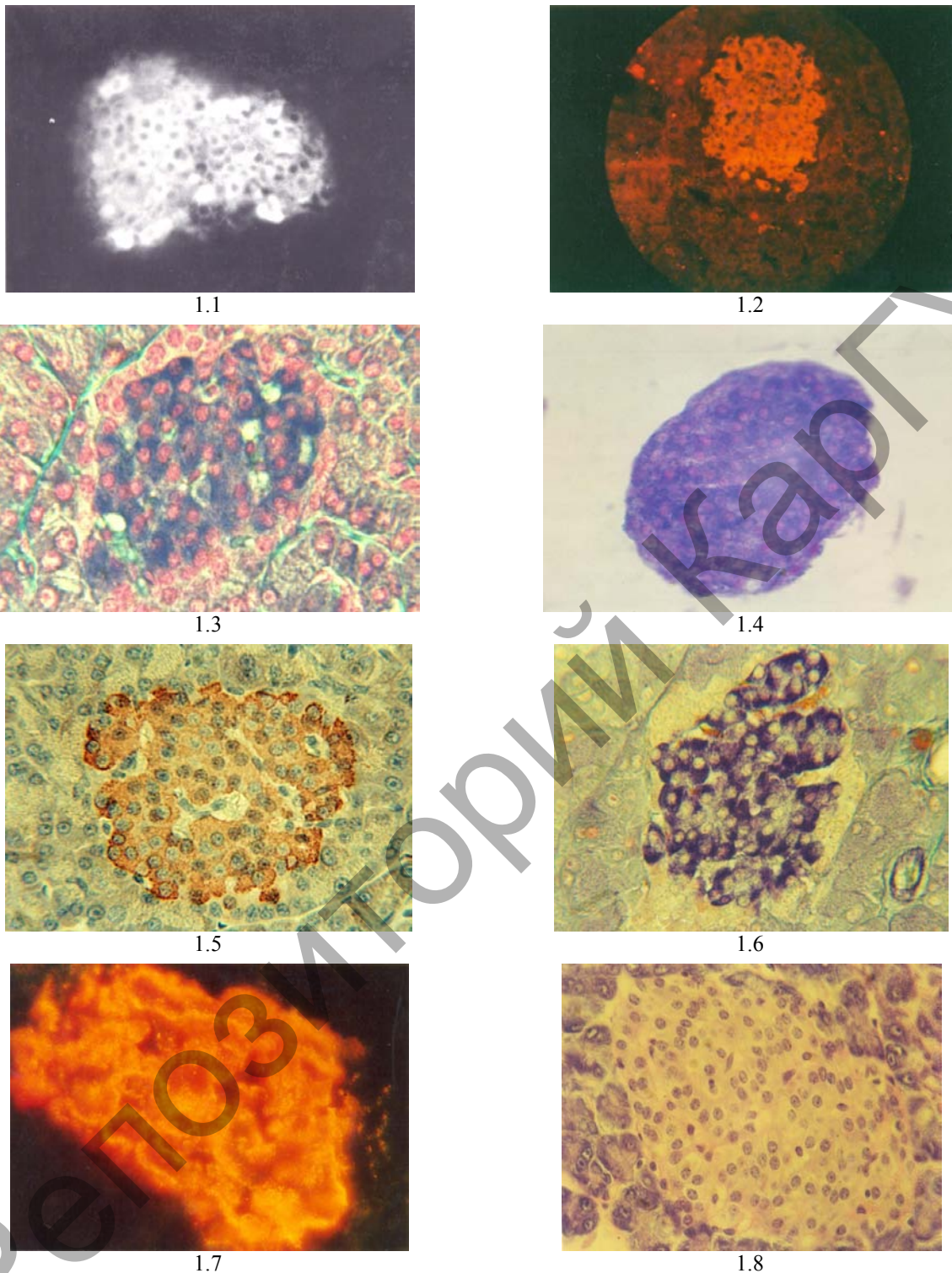
№	Pancreas tissue	Staining technologies				
		AF	IG	PS	V4	IF
1	Rats: B-cells; exocrine tissue; results staining of histostructure of islets	1.94±0,05 1.00±0,02 high	1.79±0,04 1.00±0,03 insufficient	2.02±0,11 1.00±0,08 insufficient	1.83±0,08 1.00±0,04 high	2.08±0,12 1.00±0,02 insufficient
2	Rabbits: B-cells; exocrine tissue	1.98±0,04 1.00±0,04	– –	2.07±0,12 1.00±0,07	1.96±0,05 1.00±0,03	– –
3	Rats; diabetes: B-cells; exocrine tissue	1.07±0,07 1.00±0,06	1.04±0,04 1.00±0,03	1.02±0,03 1.00±0,07	1.16±0,09 1.00±0,05	1.09±0,03 1.00±0,04
4	Density staining of exocr. tissue V4/AF, V4/IG	1.14±0,04	1.07±0,03	–	–	–

Table 2

Practical characteristics methods of insulin staining in B-cells

№	Characteristics of methods	Staining technologies				
		AF	IG	PS	V4	IF
1	Specificity for insulin staining	Specificity for staining a few hormones; for B-cells specific for insulin	Absolute specificity for insulin staining in any tissues	High specificity for staining of A-chain of insulin	High specificity for staining of A-chain of insulin	Absolute specificity for insulin staining
2	Staining of histostructure of islets and of exocrine tissue	Staining in detail; suitable for detail analysis of state of histostructure	Staining not in detail; not suitable for detail analysis of state of histostructure	Not staining of histostructure; not suitable for analysis of state of histostructure	Staining in detail; suitable for detail analysis of state of by histostructure	Not staining of histostructure; not suitable for analysis of state of histost ructure
3	Reagents	Reagents for staining are produced by many firms	Kits for insulin staining are produced by many firms; method is widely used as universal for staining many hormones	Diethylpseudoisocyanine for staining is produced only by SERVA	V4 is produced by a few firms	Anticorps are produced by a few firms as by research laboratories
4	Practical using	Long time stored histological paraffin sections of tissue	Long time stored histological slides	Slides stored a short time (0,5–1 h)	Long time stored histological slides	Slides stored a short time (0,5–1 h)

Fluorescent methods showed more high deviations of value of parameter K that can be explained by more wide fluctuation intensity of fluorescence in various islets. The highest value of parameter K was obtained at measurement intensity of staining in B-cells of complex zinc-insulin by Dithizon slides (Fig. 1.7).



- 1.1 Intact pancreatic islet. Insulin. Intensive fluorescence. Immunofluorescent staining method; 5×40;
 1.2 Intact pancreatic islet. Insulin. Red fluorescence of A-chain of molecule of insulin. Diethylpseudoisocyanine staining method; 3×40;
 1.3 Intact pancreatic islet. Insulin. Dark blue color of insulin. Victoria-4 staining method; 7×40;
 1.4 Isolated intact pancreatic islet. Dark blue color of insulin. Victoria-4 staining method; 7×40;
 1.5 Intact pancreatic islet. Insulin. Brown color of insulin. Immunohistochemical staining method; 7×40;
 1.6 Intact pancreatic islet. Insulin. Dark violet color of hormone. Aldehyde fuchsin staining method; 7×40;
 1.7 Intact rabbit's pancreatic islet. Insulin-Zn⁺² red coloring complex. Staining by Dithizon; 7×40;
 1.8 Intact pancreatic islet. Insulin. Hematoxylin and eosin staining method; 7×40.

Figure 1

High fluctuations of absorption in sections painted by Victoria-4 comparatively with AF and IG methods are obviously caused besides the following reasons: 1) evidently more intensive staining of other structures of pancreatic islets as wall of blood vessels, nucleus, connecting tissue and of exocrine tissue that result more intensive absorption of comparatively with IG and AF methods; 2) density of staining of exocrine tissue is more high too comparatively with AF and IG methods and approximately same, as well as using staining by Hematoxyline and Eosin (Table 1; Fig. 1.3–1.6, 1.8).

It was reported that a possible explanation for the ability of B-granules to react with V4 past oxidation only determined by structure of insulin. Oxidation result dissociation of disulfide bonds between two chains of molecule of insulin. It is suggested that reactivity of B-cells with V4 is dependent on the staining of oxidised A-chain of insulin. The sulphonic acid groups in the A-chain give conditions favourable for staining by V4 [10].

Analysis of characteristics of methods of insulin staining (Table 2) showed that V4 and AF methods are more preferable for estimate as of insulin content as state of histostructure of pancreatic islets and exocrine tissue. Chemicals specific of V4 method is more high comparatively with AF method.

Immunofluorescent staining method [IF]. We have obtained same results of staining by IF as using of sections of pancreas tissue (Fig. 1.1–1.3). IF is high specific method for revealing of Insulin in B-cells. Decreasing of Insulin content in B-cells of islets past action direct action of Streptosotozin was evidently demonstrated by this method (Fig. 1.3).

Diethylpseudoisocyanine chloride fluorescent method [PS], a high specific for revealing A-chain of molecule of Insulin, showed same result comparatively using of sections of Pancreas tissue (Fig. 1.4–1.6). Time for staining of sections in 0,4 % solution of Diethylpseudoisocyanine was reduced from 20 min till 15 min as was reduced time for washing of sections past staining procedures. This method showed marked decreasing of Insulin content in damaged B-cells (Fig. 1.5, 1.6) in compared with intact.

Aldehydefucshine method showed analogical results. A significant differences are revealed of state of histostructure as of Insulin content in damaged isolated islets comparatively with intact (Fig. 1.8). Aldehydefucshine method [AF] contrary to IF and PS is not belong to high specific because colours other hormones too. But for pancreatic B-cells not contained other hormones AF is specific for Insulin.

Discussion

Analysis of results showed that using of histological and histochemical methods for staining of sections of isolated pancreatic islets have similar or equal to similar results obtained in pancreas tissue past staining by same methods. Fluorescent histochemical methods as Immunofluorescent reaction for Insulin as method using of Diethylpseudoisocyanine are more sensitive and identify the very low concentrations of investigated substances as 10^{-7} – 10^{-8} , that has been confirmed by our results. Meanwhile both these methods have a common fault: histological sections past completing of staining procedures are not permanent and must be investigated within short time. Both methods are belong to high specific for staining of Insulin or of A-chain of molecule of Insulin. These methods are more precise for measuring intensity of insulin staining in B-cells because no other structure of islets are stained.

More suitable for practical using is Aldehydefucshine technic. Histological sections of pancreas tissue as of isolated islets stained by this method are permanent and can be stored for a long time. Aldehydefucshine method is not belong to high specific for Insulin staining. It is known that some pituitary hormones can also be stained by Aldehydefucshine method. Meanwhile for pancreatic islet's B-cells this method you can be measured as specific for insulin because the other hormones in B-cells are not synthesized. Method Victoria 4R is high specific for Insulin and as Aldehydefucshine technic gives an opportunity to obtain permanent histological sections. Quantitative estimation of insulin content in stained sections is based on measuring of absorbed by B-cells of light. However, both of these methods are belong to histological methods too and result staining not only of Insulin, but also other structures of B-cells which absorbed light as Insulin. Therefore, results of estimation of Insulin content in the B-cells by measuring of absorbance is not so precise as using fluorescent histochemical methods for Insulin staining.

We used significantly reduced time for fixation of Islets in Bouin from 24 h for pieces of pancreas tissue up to 15–30 min for isolated Islets. Time for staining of sections of isolated islets by Diethylpseudoisocyanine was reduced to 15 min comparatively with 20 min for sections of pancreas tissue.

Conclusions

1. V4 and AF methods evidently are more suitable for to estimate state of histostructure of pancreatic islets and of exocrine tissue, not only the content of insulin in B-cells.
2. V4 method is more specific for staining of insulin comparatively with AF method.
3. V4 method is more precise for quantitative estimate of the insulin content in B-cells comparatively with Pseudoisocyanine and Immunofluorescent methods and less precise in compared with Immunohistochemical and Aldehyde-fuchshine methods.

Acknowledgement

Authors are thankful to Prof. G.G.Meyramov, Prof F.Wohlrab (Leipzig, Germany), Dr. O.E.Grundemann ("FERAK", West Berlin), to Mr. K.Niethammer and Mr. W.Manneck ("MERCK", Darmstadt, Germany) for free substance Dimethylnaphtylmethan (Victoria Blue, c.i.42563).

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Инсулин бояғышының «Виктория-4» гистохимиялық әдісі

Мақалада «Victoria-4» гистохимиялық әдіс (мәліметтер) деректері иммунофлюоресцентті, иммуногистохимиялық, жаңа псевдоизоцианил және альдегидфуксинды басқа гистохимиялық әдістер қолдану нәтижелерімен салыстырмалы тұрғыда берілген. Авторлардың көрсетуімен бұл әдіс жоғарыдағы әдістердің болу тығыздығының сандық бағасының дәлдігін беруінде жеткіліксіз болғанымен, инсулинге қатысында спецификалық артықшылығымен ерекшеленеді. Бұл әдістің құндылығы панкреатит аралшарының әр түрлі құрылымдарын сапалы бояу негізінде В-жасушалардағы инсулинді сапалы анықтаумен қатар, аралшалардың гистоқұрылымдық күйін бағалау мүмкіндігін береді.

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«Виктория-4» гистохимический метод окраски инсулина

В работе приведены данные использования гистохимического метода Victoria-4 в сравнении с результатами применения других гистохимических методов: иммунофлюоресцентного, иммуногистохимического, псевдоизоциани нового и альдегидфуксинового. Авторами показано, что данный метод, являясь специфичным в отношении инсулина, уступает по точности при количественной оценке плотности окраски перечисленным выше методам, но выигрывает в стоимостном отношении. Его преимуществом также является достаточно качественная окраска различных структур панкреатических островков, что дает возможность точнее оценивать состояние гистоструктуры островков, помимо определения содержания в В-клетках депонированного инсулина.

Репозиторий КАРГУ