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Histological and Histochemical Methods for Staining of Insulin: A Comparative Analysis

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Short Communication

Various histochemical and histological methods are used for analysis of the state of histostructure and of insulin content in pancreatic B-cells. Aim of work: a comparative analysis of the results of using of 3 staining technologies refined and detailed by us: Aldehyde fucshine, AF, fluorescent diethylpseudoisocyanine method, PS and Dimethylnaphtylmetan, V4 (Victoria 4R, color index 42563). Intensity of fluorescence of A-chair of insulin (PS) measured using of histofluorimetric complex [1]. Calculation of parameter FL= IF1/IF2 where IF2–intensity of fluorescence of intact B-cells (as 1.00) and IF1–B-cells in diabetes. Density of color (AF and V4), parameter DC= DC1/DC2 where DC2-density of color of intact B-cells (as 1.00) and DC1-B-cells in diabetes.

Results

Aldehyde-Fucshine Method

Violet granules in cytoplasm of B-cells correspond to deposited form of insulin [2,3]. Intensity of color of cytoplasm of B-cells directly correspond to insulin content in cytoplasm]. Staining procedures modified by us.

a. Deparaffinization of sections in xylol in 3 portions of xylol;

b. Alcohol 100^0-5 min,

c. Alcohol 100^0-5 min,

d. Alcohol 80^0-5 min,

e. Water- 5 min,

f. Oxidation 0,5-2 min oxidation solution: 5 ml of 5% H_2SO_4+5 ml 2,5% solution of $KMnO_4$+30 ml bidistilled water at $+28^0$ Celsius -2 min,

g. 2% solution of oxalic acid-rinse until discoloration,

h. Distil. water-5 min,

i. Aldehyde fucshine (“MERCK”; “SERVA”)-5-7 min,

j. Dcidified alcohol 70^o №1 – differentiate,

k. Acidified alcohol 70^o №2 – differentiate,

l. Halmy’s solution-1 min,

m. Distil. water-5 min,

n. Distil. water №2-5 min,

o. Alcohol 100^0-5 min,

p. Alcohol 100^0 №2-5 min,

q. Xylol - 5 min,

r. Xylol №2 - 5 min,

s. Balm,

Result:

Violet color of insulin in B-cells (Figure 1.1) (intact animals; 1.4 in animals with diabetes).

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 method [4,5]. Staining procedures. Preparing of staining solution: 0,4% water solution of Diethylpseudoisocyanine (SERVA, Germany). Staining procedures:

a. Deparaffinization of sections in xylol;

b. Alcohol 90^0,80^0,70^0 1 min in each;

c. Washing in cold water;

d. Oxidation 0,5-2 min; Oxidation solution: 5 ml of 5% H_2SO_4+5 ml 2,5% solution of $KMnO_4$+30 ml bidistilled water at $+28^0$ Celsius;

e. Washing in cold water;

f. 5% solution of oxalic acid -5 sec;

g. Washing in 2 portions of cold water;

h. 0,4% cold solution of Diethylpseudoisocyanine - 20 min in refrigerator at +4^{0}Celsius;

i. Washing in cold water 5 min;

j. Store in refrigerator 1,5-3h. Result Intensive red fluorescence of insulin in B-cells (Figure 1.2) intact animals; 1.5 in animals with diabetes) (Table 1).

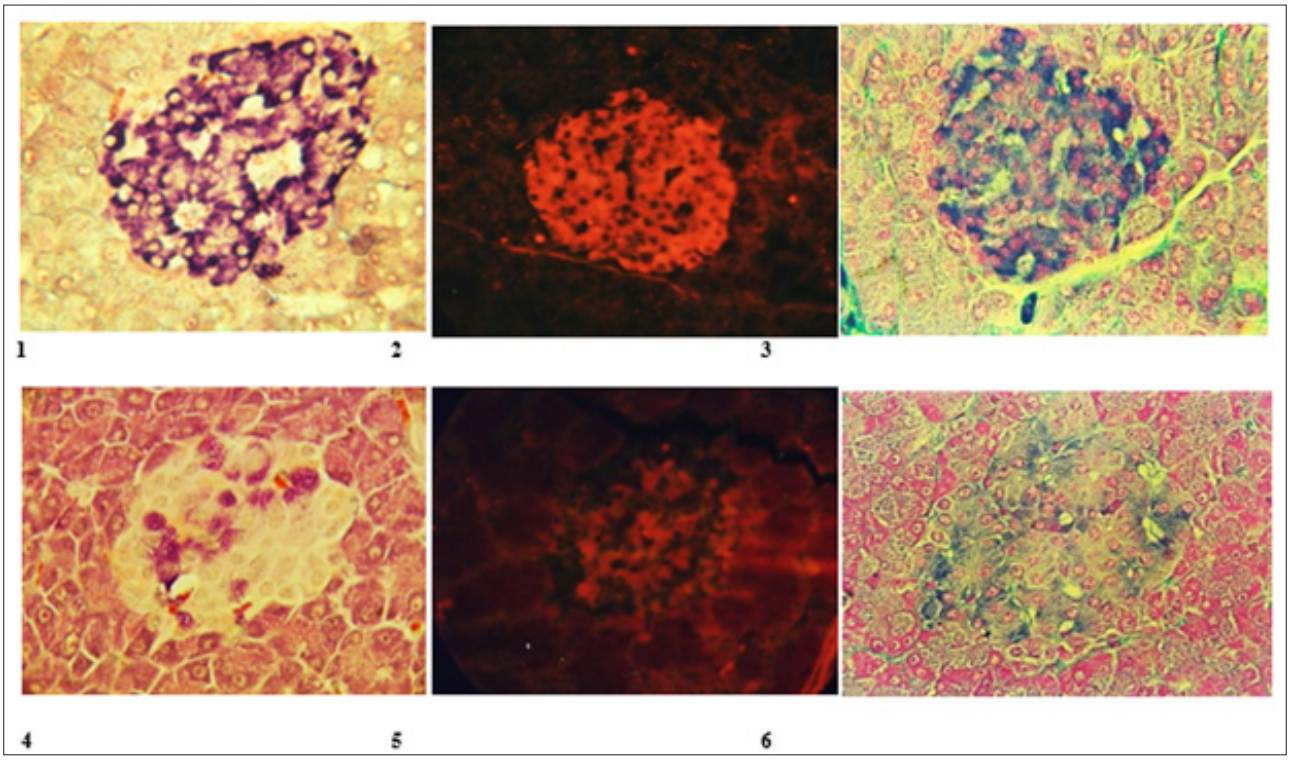

Figure 1: 1-3 - intact animals; 4-6 - animals with diabetes.

Table 1: Result Intensive red fluorescence of insulin in B-cells (Figure 1.2) intact animals; 1.5 in animals with diabetes).

№	Staining Technology	Intact	Diabetes	Positive Properties of Method	Negative Properties of Method
1	Aldehyde-fucshin	1.00±0.08 n=18	0.22±0.05 n=23	a good opportunity for inves-tigation of histotopography of in-sulin in B-cells and of state of histostructure; precise method for investigate of insulin content	
2	Diethylpseudoisocyanine	1.00±0.03 n=20	0.12±0.02 n=25	minimal fluctuations of indicators of insulin content; high sensitivity and specifity for insulin; precise method for investigate of insulin content	only fresh staining secti- ons of tissue can be used.
3	Dimethylnaphtylmetan	1.00±0.10 n=24	0.29±0.06 n=19	high specifity for insulin; prefe-rable for investigation of histostru-cture of islets	marked fluctuations indi-cators of insulin conent

Victoria Blue 4R Method Staining of Insulin (V4R)

Diphenylnaphthylmetane, colour index 42563; MERCK, Germany; FERAK,Germany). It was showed (8) that V4R 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 [6,7]. Staining procedures:

a. Deparaffinization of sections,

b. Washing in cold water a few min,

c. Oxidation 3-5 min(oxidation solution: 0,3% $KMnO_4$ 50 ml+0,3% H_2SO_4 50 ml; wash sections,

d. Place sections in 2-5% water solution of sodium bisulphate - 1 min; wash sections,

e. 70^0 alcohol-1 min,

f. Stain in staining solution (96^0 alcohol 100 ml+Victoria Blue 4R - 1g) 15 min - 2h; wash sections,

g. Stain on 0,5% water solution of Phloxine 30-120 sec.; wash sections;

h. 5% water solution of phosphor wolframic acid 1-2 min; wash section in water;

i. Stain in 0,5% water solution of Light Green 1-2 min;

j. Dehydratation in 96^0 alcohol;

k. Balm. Result: blue color of insulin in B-cells; red color of A-cells (Figure 1) (intact animals; 1.6 in animals with diabetes).

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