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Long time prolonged elimination of zinc-insulin complex from B-cells not result dysfunction of cells

Authors showed that almost complete elimination of zinc-insulin complex from cytoplasm of B-cells caused by 3 days prolonged administration of Glibenclamide to animals accompanied by complete disappearing of insulin and zinc-ions from B-cells. Next 6–7 days free of using of Glibenclamide result parallel complete recovery of amount of insulin and zinc in B-cells without any changes of histostructure of islets and function of B-cells.

Key words: rats Vistar, histology, insulin, B-cells, dissociation of complex, secretion, pancreatic islet, histostructure.

Pancreatic B-cells of many sorts of animals and of human contained a large amount of zinc-ions which take part in process of forming deposited form of insulin in cells which concentrated in B-granules of cells. In pancreas tissue B-cells contain large amounts of zinc. The major role of zinc is the binding of insulin in hexamers [1]. Zinc ions and insulin create a hexameric, crystalline structure, comprising 2 zinc ions and 6 insulin molecules, which is stored in the secretory granules until secreted in response to metabolic demands [2]. Zinc in B-cell secretory granules is involved in the storage and stabilization of the insulin hexamere in B-cells [2, 3]. Zinc ions appear to play important significance in process of microcrystallization of the precipitated insulin granules. May be it is advantage in condensing the stored hormone [2].

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It is known that prescription of Sulphorea result elimination of complex insulin-zinc from B-cells [4]. It was showed that 1 week past of partial or almost complete elimination of zinc-insulin complex from B-cells, function of cells are restored. It is not investigated question: are eliminated from B-cells zinc and insulin, insulin only or insulin and part of zinc-ions. Previously it was reported that binding of zinc-ions in B-cells by diabetogenic or not diabetogenic chelat active chemicals result a complete binding of zinc-ions and dissociation of complex 1,5–2 h later: chemicals are removed from cells and zinc remains in the cytoplasm of B-cells [5].

Meanwhile now is not cleared what is insulin and zinc content in cytoplasm of B-cells more long time after elimination of insulin from B-cells.

Aim of work: to investigate insulin and zinc ions content as state of histostructure of pancreatic islets more long period later, as 30 days, after almost complete elimination of zinc-insulin complex from B-cells.

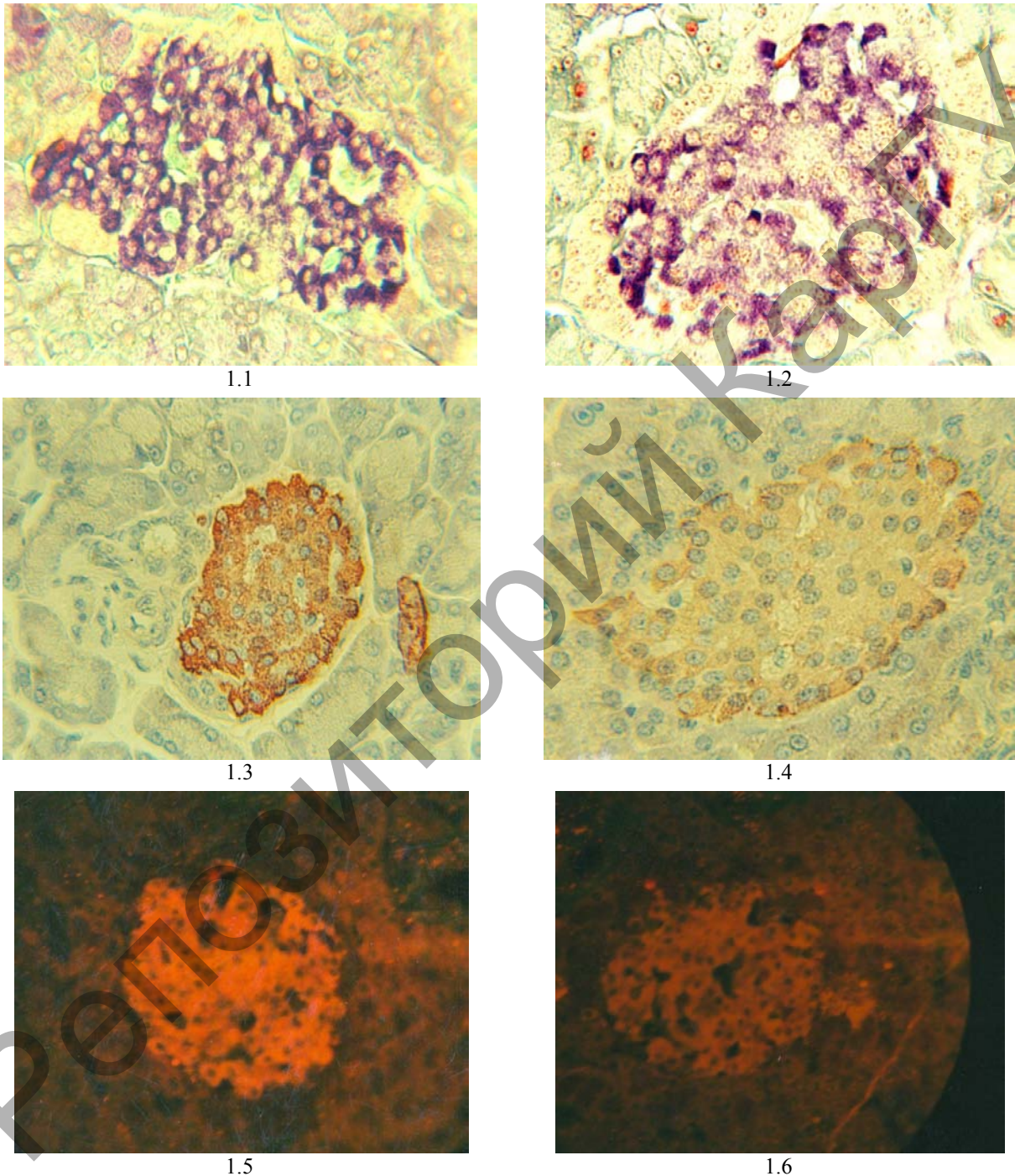
Materials and methods

26 rats Vistar 170–185 g were used. 2 % starch suspension of Glibenclamide (GB) used for peroral administration to animals in doses 10 and 25 mg/kg for 6 days 1 time daily. Blood Glucose control: 3 h, 24 h, 3 days 15 days and 30 days after past administration of GB). Histostructure of pancreas tissue and insulin content in B-cells were studied 30 days after administration of GB.

Histology. Pancreas tissue were fixed in Bouin. Sections 4–5 mcm were stained by hematoxylin and eosine; insulin staining by aldehyde fuchshine [6], pseudoisocyanine [7–9] and zinc-ions — by 8-para(toluenesulphonylamino)quinolin (TSQ) [9]. Intensity of fluorescence of insulin and of zinc-ions was measured by fluorescent histofluorimetric complex constructed by G.G.Meyramov [10]. For transmission electron microscopy samples of pancreas tissue were fixed in 2,5 % Gluthar-aldehyde. Ultrafine sections of tissue contrasted by Reynolds [11] and were investigated on electron microscope JEM-7A.

Results

Blood Glucose concentration past administration of GB. Results showed that maximal decreasing of BG concentration was observed 3 h past first administration of 10 mg/kg (-approx. 20 %) and of 25 mg/kg (-28 %). 24 h past administration there are some not authentically prevalence of BG level in comparison before administration of GB. Next period from 3rd days until 30th days BG concentrations was not changed.



- 1.1 — Pancreatic islet of intact rat. Aldehyde fuchsin; $\times 280$;
 1.2 — Pancreatic islet 3h past action of GB 10 mg/ kg. Decreasing of insulin content in B-cells. Aldehyde fuchsin; $\times 280$;
 1.3 — Pancreatic islet of intact rat. Immunohistochemistry; $\times 280$;
 1.4 — Pancreatic islet 3h past action of GB 25 mg/kg. Decreasing of insulin content in B-cells. Immunohistochemistry; $\times 280$;
 1.5 — Pancreatic islet of intact rat. Pseudoisocyanine; $\times 200$;
 1.6 — Pancreatic islet 3h past action of GB 10 mg/kg. Decreasing of insulin content in B-cells. Pseudoisocyanine; $\times 200$

Figure 1

Table 1

Blood Glucose concentration past administration of GB

	Dose	Blood Glucose concentration, mM					
		before	3 h	24 h	3 days	15 days	30 days
1	Control (intact)	5,1±0,31	5,0±0,21	4,9±0,14	5,2±0,28	5,1±0,25	5,1±0,32
2	GB, 10 mg/kg	4,7±0,22	3,8±0,16 $p < 0,05$	4,8±0,15	4,8±0,23	4,9±0,17	4,6±0,23
3	GB, 25 mg/kg	4,6±0,15	3,3±0,14 $p < 0,05$	4,9±0,12	5,1±0,29	4,8±0,15	4,5±0,13

Table 2

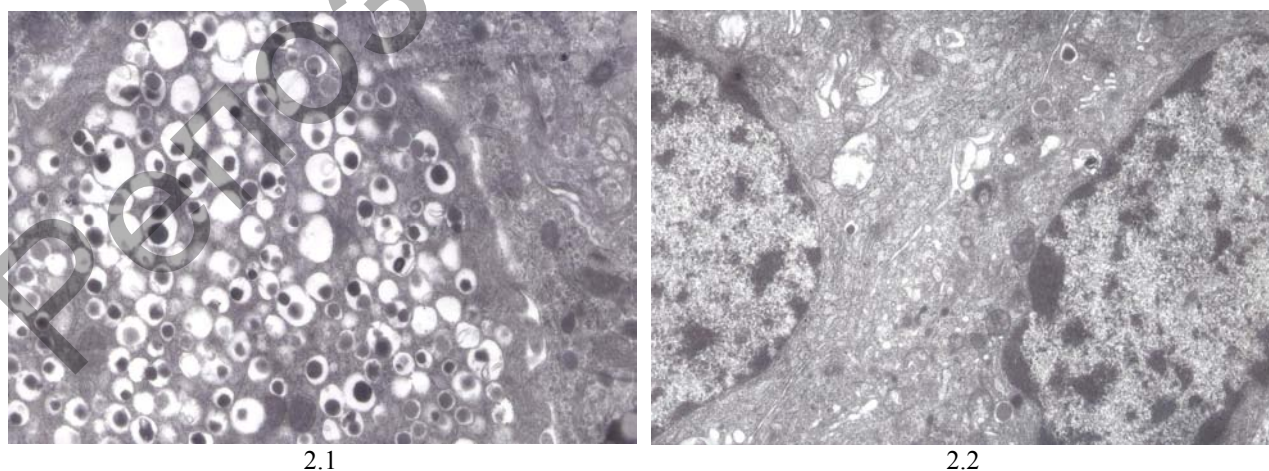
Insulin and Zinc content in pancreatic B-cells past administration of GB (parameter K)

	Condition	Insulin and Zinc content in B-cells (K)					
		before GB		3 h after GB		30 days after GB	
		insulin	zinc	insulin	zinc	insulin	zinc
1	GB, 10 mg/kg	2,04±0,05**	2,00±0,03	•1,62±0,04**	•1,71±0,03	1,94±0,06	1,87±0,05
2	GB, 25 mg/kg	1,95±0,06*	1,97±0,05	1,38±0,04*■	1,62±0,04■	2,00±0,07	1,96±0,06
3	Intact	2,02±0,05	1,98±0,06	1,96±0,07	1,94±0,08	1,97±0,04	2,02±0,05

Note. * — $p < 0,01$; ** — $p < 0,05$; ■ — $p < 0,05$; • — $p < 0,05$.

Results of estimation of insulin and zinc content in B-cells showed evident decreasing amount as of insulin as of zinc-ions 3 hours after GB administration (Table 2; Fig. 1.1–1.6; Fig. 2.1, 2.2). Decreasing is more marked past administration of 25 mg/kg — for almost 30 % comparatively with approximately 20 % past administration of 10 mg/kg. Results showed that a coincidence of results between the contents in B-cells of insulin and zinc is available only for intact B-cells and after 24 h and 30 days past administration of drug. 3 hours after administration of GB results showed more intensive decreasing of insulin content comparatively with content of zinc-ions (Table 2). Results obtained before action of GB were authentically prevailed in compared with zinc ions past administration of both doses of GB. 30 days past administration of both doses of GB insulin and zinc content in B-cells were restored completely. There are not any histological changes in pancreatic islets 30 days after action of GB (Fig. 1.2, 1.4).

We found discrepancy more marked differences between results measuring of insulin and zinc-ions content 3 h past action of GB 25 mg/kg (Table 2): approximately 30 % of insulin are eliminated from B-cells and 18–20 % of zinc-ions only eliminated contrary to parallelism of results 30 days past action of GB. It is possible to suppose that part of amount of zinc-ions after dissociation of complex is eliminated from B-cells and part remain in cells (Fig. 3).



2.1 — Intact pancreatic islet. B-cells. Large number of B-granules contained zinc-insulin complex; ×3450;
2.2 — Islet 3 h past administration to animal of GB 25 mg/kg. Marked decreasing of number of B-granules; ×2920

Figure 2. Transmission electron microscopy

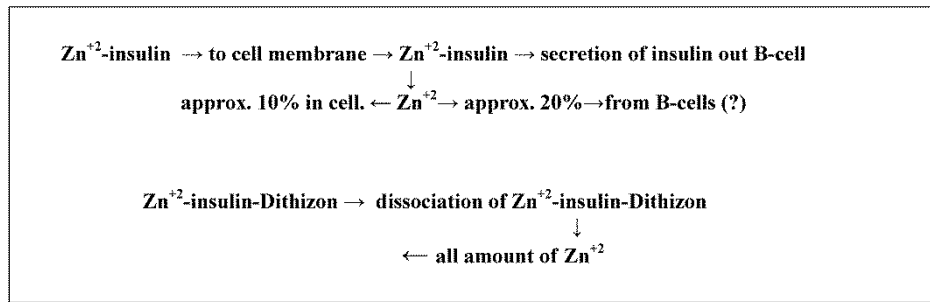


Figure 3. Dissociation of complexes Zn^{+2} -insulin and Zn^{+2} -insulin-Dithizon in B-cells

Conclusions

1. Elimination of insulin from B-cells by GB accompanied by partial decreasing of zinc-ions content 3 h after action and completely is restored 30 days later. Insulin content in B-cells reduced for approximately 30 % and of zinc-ions — for 18–20 % 3 hours after administration of 25 mg/kg of GB.

2. There are not any histological changes of histostructure of pancreatic islets 30 days past elimination of zinc-insulin complex from B-cells. Amount of insulin and zinc-ions in B-cells restored completely 30 days later.

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В-жасушалардан мырыш-инсулин кешенінің ұзақ уақыттағы элиминациясы және олардың функциясы бұзылуына катысының жоқтығы

Авторлар «Глибенкламидті» үш күндік енгізуден кейін В-жасушаларынан мырыш-инсулин кешенінің элиминациясы цитоплазмадан мырыш пен инсулиннің толықтай жоғалуына әкеліп соққандығын анықтады. Препаратты енгізуді тоқтатқаннан кейін жасушалардағы мырыш пен инсулиннің мөлшері

толықтай орнына келді. Панкреатиттік өсінділердің гистоқұрылысының жағдайында ешқандай өзгерістер байқалмады.

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**Длительная элиминация цинк-инсулинового комплекса
из В-клеток, не сопровождающаяся нарушениями их функций**

Авторами установлено, что элиминация из В-клеток цинк-инсулинового комплекса, вызванная трехдневным введением Глибенкламида, сопровождалась полным исчезновением из цитоплазмы инсулина и цинка. После прекращения введения препарата содержание цинка и инсулина в клетках полностью восстанавливалось. Никаких изменений состояния гистоструктуры панкреатических островков не выявлено, как и инсулинпродуцирующей функции В-клеток в последующем.

Репозиторий КарГУ