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Cysteine Prevents the Development of Experimental Diabetes Induced by Zinc-Binding Substances

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In experimental rabbits, cysteine injected intravenously in a dose of 1000 mg/kg temporarily bound zinc in β cells and prevented the formation of chelate zinc complexes in response to subsequent injection of diabetogenic zinc-binding substances that induce cell destruction. Injection of cysteine to animals was associated with a sharply negative reaction to zinc in β cells, which attests to blockade of zinc ions. Injection of cysteine few minutes after dithizone and formation of zinc—dithizone complex was followed by displacement of dithizone from the complex and prevented the development of diabetes in most animals. The most plausible mechanism of preventive effect of cysteine is the formation of 2:1 zinc—cysteine complex in β cells with possible fixation of Zn atom between sulfur atoms from SH groups of two cysteine molecules.

Key Words: *β cells; cysteine; sulfhydryl groups; diabetogenic zinc-binding substances (DZB); 8-para-toluene sulfonamide quinoline (TSQ)*

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Pancreatic islet β cells of rabbits, mice, dogs, cats, hamsters, horses, and humans contain an appreciable amount of zinc [1,3,5] that is involved in insulin biosynthesis and formation of its depot (Zn^{2+}-insulin complex) in β cells [4]. Diabetogenic Zn-binding substances (DZBS) [1-3] form complex salts (chelates) with zinc in β cells, which leads to destruction of β cells within 15-30 min and development of type 1 diabetes. Cysteine amino acid can bind heavy metal ions with the formation of complexes and is characterized by high affinity to zinc; stability constant is higher than for Zn complexes with DZBS (logarithm of stability constant 17.1-18.2) [1].

We studied the possibility of preventing DZBS-induced diabetes with cysteine. To this end, we evaluated cysteine capacity to bind zinc in β cells for preventing destruction of β cells and development of diabetes.

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MATERIALS AND METHODS

The study was carried out on random-bred rabbits (*n*=27; 2350-2750 g) in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Group 1 animals (*n*=6) were injected with cysteine in a dose of 984-1020 mg/kg. In 3 rabbits, the reaction to zinc in β cells was studied in 10 min using TSQ, in others, the dynamics of glycemia and histological structure of the pancreas in 8 days were studied. Group 2 animals (*n*=7) received a diabetogenic dose of dithizone (47.8-50.7 mg/kg), after which the dynamics of glycemia and histological structure of the pancreas in 9 days postinjection were studied in 4 rabbits; in others, the content of Zn—dithizone complex in β cells was studied in 10-15 min after injection. Group 3 rabbits (*n*=8) received cysteine (970-1010 mg/kg) and 10 min later — dithizone (49.6-51.3 mg/kg), after which the dynamics of glycemia and the histological structure of the pancreas in 9 days postinjection were studied in 5 animals and the content of zinc—dithizone complex

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in β cells was studied in 10 min after dithizone injection in 3 rabbits. Group 4 animals (*n*=6) were injected with dithizone (46.3-48.5 mg/kg) followed in 6-7 min by cysteine injection (1000-1020 mg/kg), after which the dynamics of glycemia and histological structure on day 9 were studied in 4 rabbits and the content of zinc—dithizone complex in β cells was studied in 10-15 min after cysteine injection in the rest 2 animals.

Histological and histochemical methods were used in the study: aldehyde fuchsin method, insulin-specific fluorescent pseudoisocyanine method [6], and dimethylnaphthylmethane method (# 42563, Merck) (Victoria-4R) [7,13]. The content of zinc and insulin in β cells [8] was evaluated by fluorescence index (IF1/IF2, where IF1 was fluorescence intensity of intact β cells (mA), taken for 1, and IF2 was fluorescence of β cells after cysteine injection) in accordance with the direct relationship (the higher fluorescence intensity, the higher zinc content), and the result was expressed in rel. units. Histochemical study of zinc content in β cells was carried out with the use of highly specific fluorescent reaction with TSQ fluorochrome [10].

The results were processed by ANOVA using Student's *t* test. The data were presented as the means with quadratic deviations. The differences were significant at $p \leq 0.05$.

RESULTS

A short-term elevation of blood glucose in comparison with the initial level was observed in group 1 animals injected with cysteine during the first hours after injection. In 4-6 days, blood glucose returned to normal level (Table 1). Injection of cysteine did not affect histological structure of the pancreatic islets and insulin content in β cells. In preparations of the pancreas, numerous β-cell islets with normal levels of deposited insulin were observed. Injection of dithizone in the diabetogenic dose to group 2 animals led to a significant elevation of blood glucose level as early as on day 2 (Table 1) and induced histological changes in the islets typical of experimental type 1 diabetes: necrosis and destruction of the majority of β cells, decrease in insulin content, and drastic decrease in the number of islets (Fig. 1, *b*). Group 3 animals (cysteine+dithizone) developed slight glycemia, but glucose level returned to normal in 4-6 days (Table 1). Six of seven rabbits in this group developed no diabetes; only one rabbit developed mild diabetes (hyperglycemia 7.5 mmol/liter) and minor histological changes in 30-35% islets; no histological changes were detected in the rest animals, and their glucose levels corresponded to those in β cells of intact animals (Table 2; Fig. 1, *a*).

The formation in β cells of a zinc—dithizone complex destroying β cells after preinjection with cysteine attracted our special attention. In the islets of animals injected with dithizone alone, numerous bright-red granules of the complex were seen in the cytoplasm of β cells, while after preliminary injection of cysteine followed by dithizone, the content of these granules was much lower (0.16±0.02; Table 2; Fig. 1, *c*, *d*). Special experiments were carried out in order to rule out possible interaction of cysteine with dithizone outside the pancreatic islets: group 4 animals were injected with cysteine (975-1010 mg/kg), after which histochemical reaction for zinc in β cells was carried out on sections of frozen pancreatic tissue. Sharply negative reaction to zinc was observed in β cells of all animals 10 min after injection (Fig. 2, *a*, b), which suggested that cysteine blocked zinc in β cells and prevented its binding to dithizone. This result suggests that the protective effect of cysteine is realized at the level of β cells.

Affinity of cysteine for zinc is high (17.1-18.2) and considerably surpasses that of dithizone and 8-hydroxyquinoline derivatives. For this reason, we carried out additional experiments to confirm the fact that zinc blockage in β cells was the key event in the protective effect of cysteine. Group 4 rabbits (*n*=6) received injection of a diabetogenic dose of dithizone; in 7-8 min, *i.e.* when the zinc—dithizone complex had formed in β cells, cysteine (965-990 mg/kg) was administered. In animals injected with dithizone alone, numerous granules of the zinc—dithizone complex filled the cytoplasm of β cells as soon as just 4-5 min after injection (Fig. 2, *c*), while in animals injected

TABLE 1. Dynamics of Blood Glucose Level in Rabbits (mmol/liter; *M*±σ)

Experimental conditions	Initially	Time after injection				
		3 h	24 h	2 days	4 days	8 days
Intact animals	5.0±0.4	—	5.6±0.5	5.3±0.4	4.9±0.2	5.1±0.2
Cysteine	5.2±0.4	6.4±0.5	5.6±0.3	5.3±0.3	5.4±0.4	4.8±0.3
Dithizone	4.9±0.3	3.2±0.3	8.4±0.5	12.2±0.7	14.1±0.3	17.8±1.2*
Cysteine+dithizone	5.3±0.3	7.2±0.5	6.3±0.4	6.1±0.7	5.9±0.6	5.2±0.1*

Note. *$p \leq 0.005$ in comparison with the initial level.

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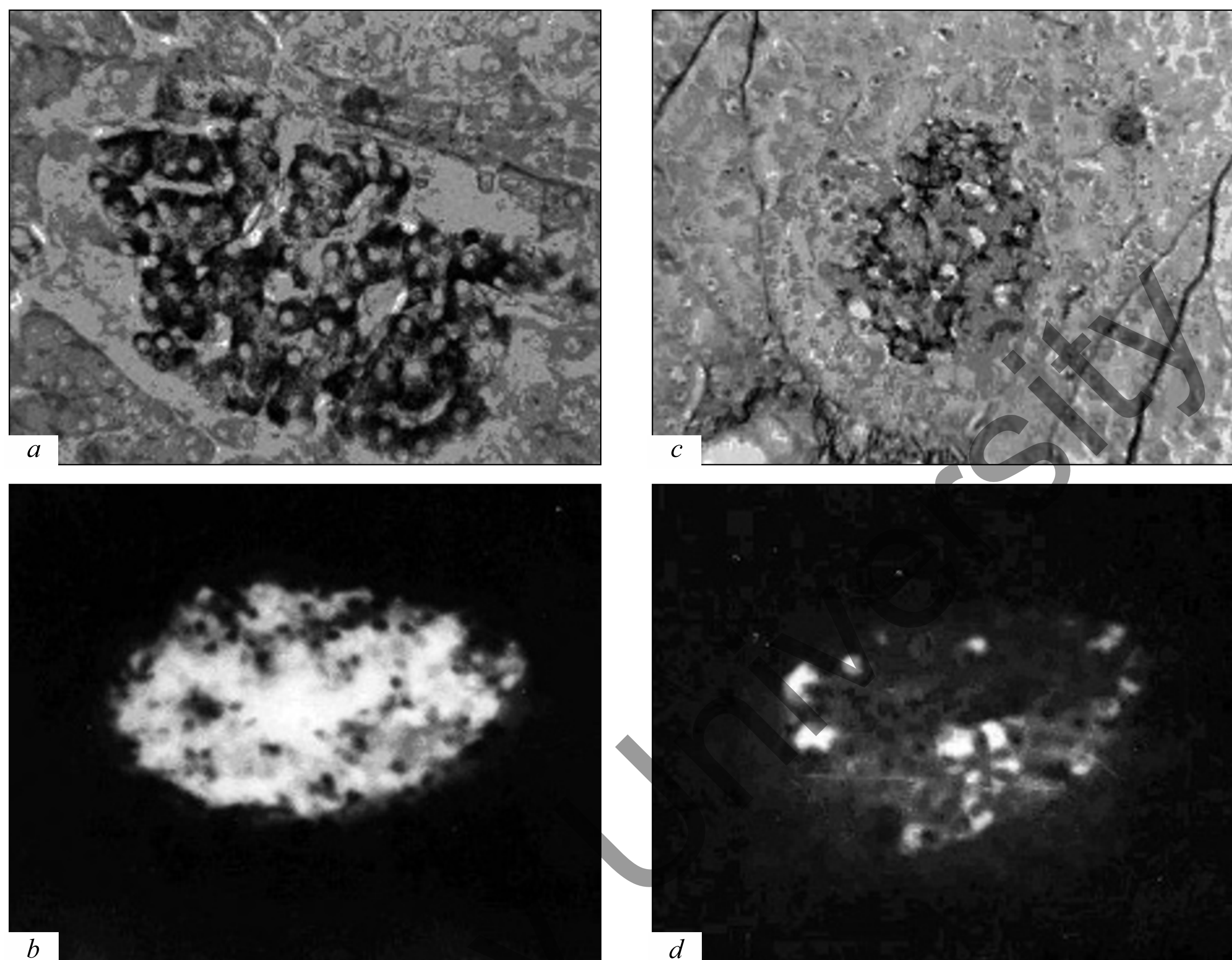

Fig. 1. Cysteine effects on rabbit pancreatic β cells. Aldehyde fuchsin staining, ×280 (*a*, *c*); frozen section of the pancreas, fluorescent microscopy, ×140 (*b*, *d*). *a*) Pancreatic islet after injection of cysteine (950 mg/kg) followed (after 10 min) by diabetogenic dose of dithizone (47.8 mg/kg): unchanged histological structure of the islet and insulin level in β cells; *b*) pancreatic islet of intact rabbit: positive TSQ reaction to zinc in β cells; *c*) pancreatic islet after diabetogenic dose of dithizone: destruction of the majority of β cells and low insulin level; *d*) pancreatic islet after cysteine (980 mg/kg): negative TSQ reaction to zinc in β cells.

with cysteine several minutes after dithizone, just solitary granules of the complex were seen in β cells (Fig. 2, *d*). These results indicated that cysteine not only prevented, but also displaced DZB substances from their complexes with zinc, thus preventing cell destruction and development of diabetes in cases when this could take place no later than 8-10 min after the complex formation, as the presence of the complex in β cells for a period longer than 15-30 min [1,3] led to irreversible changes.

Among complexes formed by 8-hydroxyquinoline derivatives with 1:1 component ratio, the strongest are those with stability constant of 7.6-9.4; complexes of azaoxine isomer with stability constant of 5.8 to 6.7 are significantly less stable and toxic [1,5]; complexes with stability constant 8.5 are the most toxic of 8-hydroxyquinoline derivatives [5]. Pentagonal rings forming during metal-chelator interactions, are more stable, while formation of a complex with involvement of a sulfur atom adds stability to quadragonal chelates [5]. Complexes with zinc atom fixed between two atoms of nitrogen, sulfur, or oxygen in the chelator molecule are the most stable. Only 8-hydroxyquinoline derivatives with a hydroxyl or another radical containing nitrogen, sulfur, or oxygen atoms in position 8 are diabetogenic (Fig. 3). Zinc atom in these cases is fixed between sulfur and oxygen atoms in position 8 and between nitrogen or oxygen atoms in positions 1 or 2. Extraction of the radical from position 8 leads to complete loss of diabetogenic activity by the substance [5].

Cysteine is characterized by high affinity for heavy metal ions, including zinc ions, and binds them with the formation of compounds not damaging the cells. High stability constant of its complex with zinc is superior to that of its complexes with 8-hydroxyquinoline derivatives and dithizone. Amino acids form complexes with metals (including zinc), and these complexes often have SH groups in their structure.

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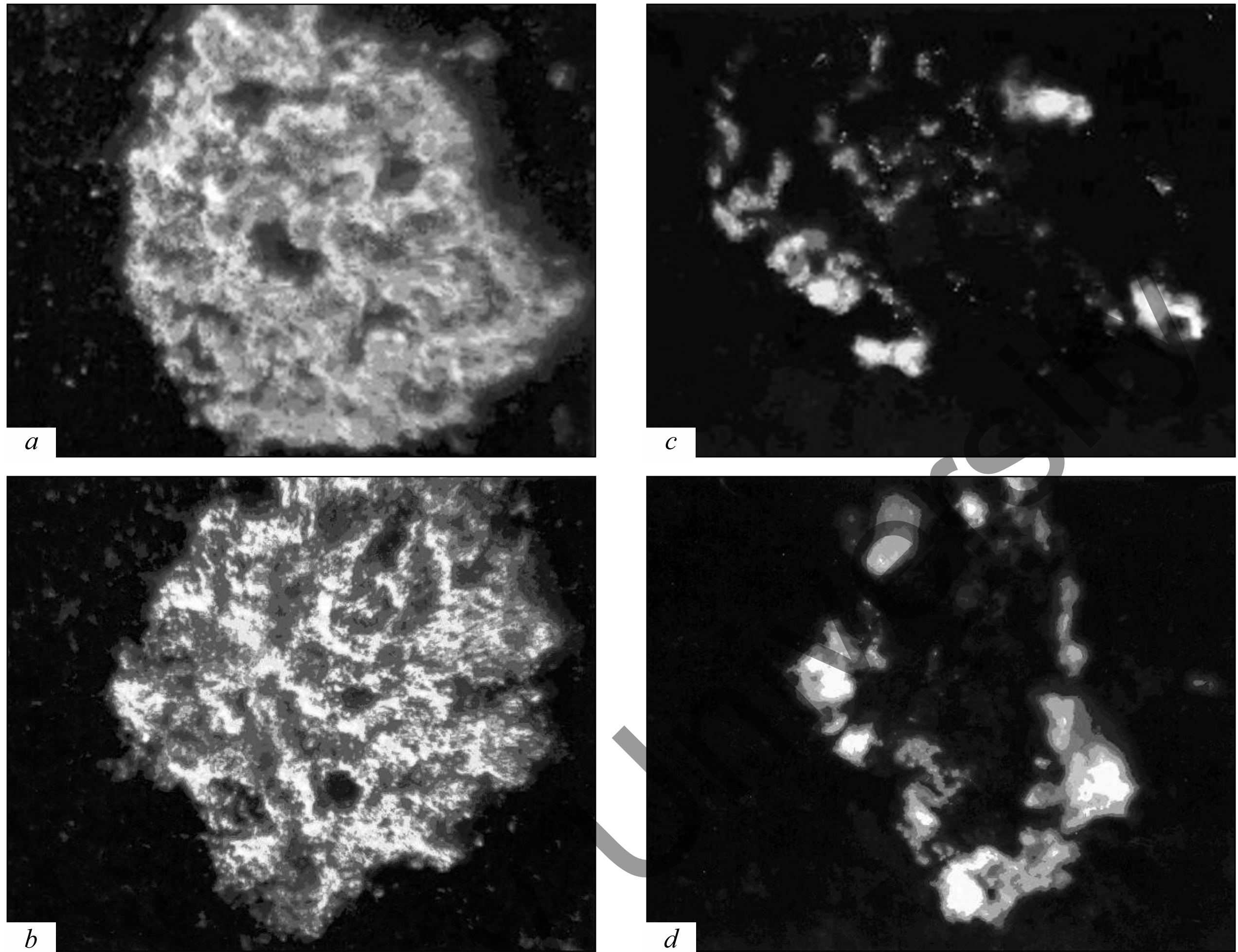

Fig. 2. Cysteine and dithizone effects on detection of zinc in β cells. Frozen section of the pancreas, dark field microscopy, ´280. *a*) Pancreatic islet of intact rabbit: positive dithizone reaction to zinc in β cells; *b*) pancreatic islet of intact rabbit 4 min after dithizone injection: cytoplasm densely packed with zinc—dithizone complex granules; *c*) pancreatic islet after injection of 980 mg/kg cysteine followed by dithizone (48.8 mg/kg): negative dithizone reaction to zinc in β cells; *d*) pancreatic islet 5 min after dithizone followed by cysteine (965 mg/kg): solitary granules of zinc—dithizone complex in β cells.

Addition of reduced glutathione and histidine (1000 mg/kg) reversibly blocks zinc in β cells, preventing its binding to dithizone and development of diabetes in all experimental animals [9,11]. Oxidized glutathione has no SH groups, and its injection to animals in the same dose does not lead to its binding zinc and does not prevent diabetes in animals [9]. Hence, the absence of SH groups in glutathione molecule is associated with loss of their capacity to prevent diabetes caused by DZBS [9]. Cysteine is a bidentate ligand and trough SH groups forms 1:1 complexes (zinc atom fixed between sulfur atom from one side and by nitrogen atoms from amino group or oxygen from carboxyl group from the other side) or 2:1 complexes (zinc atom fixation between two sulfur atoms of SH group from two cysteine molecules, similarly as glutathione) with metals [12]. Higher stability of 2:1 zinc—chelator complex is explained by the fact that zinc atom is fixed between two sulfur atoms, for which zinc affinity is the highest and higher than its affinity for oxygen. Moreover, two cysteine molecules with a greater sum of double bonds are involved in conjugation. The stability of 2:1 complex is determined by the chelator affinity for metal and by two more characteristics of the chelator and metal: presence of additional radicals in para-positions of chelator molecule, particularly in parts of the molecule contacting with its part reacting with metal ions, and the molecule diameter. If the diameter is very little, the ring cannot form: zinc atom radius is 0.74 nm and it is located between beryllium (0.31 nm) and rubidium (1.49 nm) atoms, which is quite enough for the formation of a ring [1]. High stability of 2:1 zinc—dithizone complex is additionally due to elongated shape of dithizone molecule and location of two phenol rings at the molecule terminals, due to which nitrogen and sulfur atoms easily come close

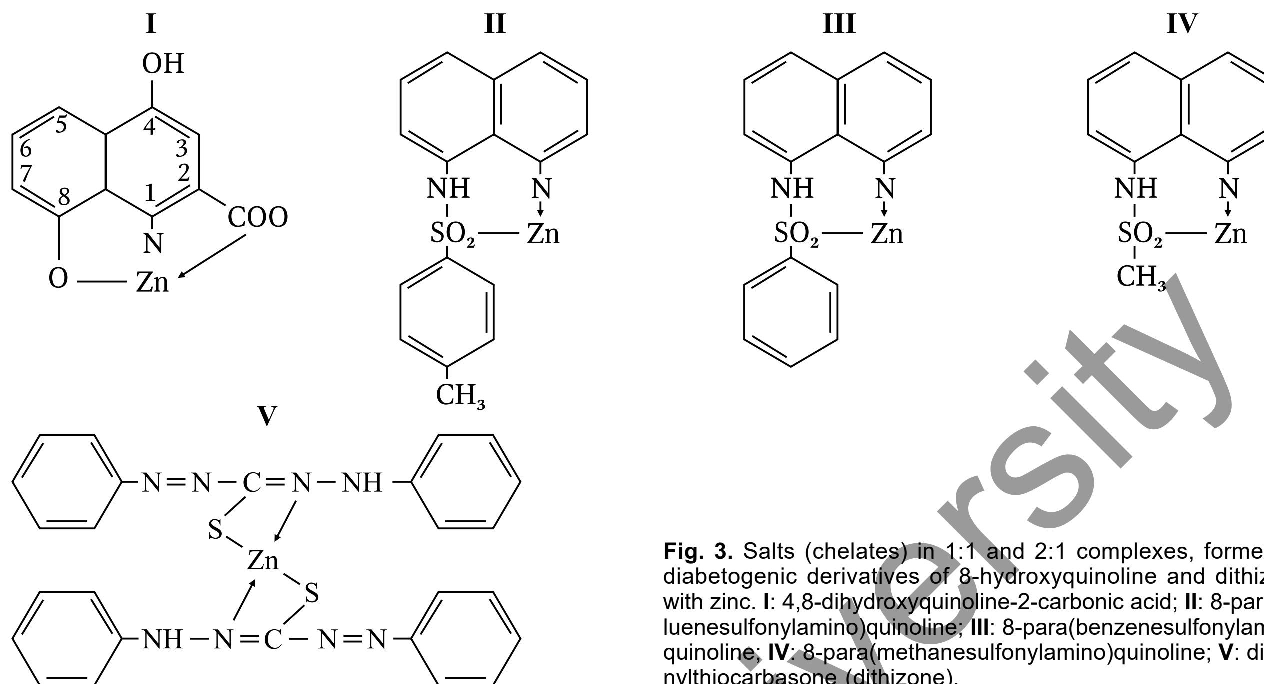

Fig. 3. Salts (chelates) in 1:1 and 2:1 complexes, formed by diabetogenic derivatives of 8-hydroxyquinoline and dithizone with zinc. **I**: 4,8-dihydroxyquinoline-2-carbonic acid; **II**: 8-para(toluenesulfonylamino)quinoline; **III**: 8-para(benzenesulfonylamino) quinoline; **IV**: 8-para(methanesulfonylamino)quinoline; **V**: diphenylthiocarbasone (dithizone).

to zinc atom (Fig. 3). Zinc atom in this case is fixed between nitrogen and sulfur atoms, for which zinc is highly affine. In addition, zinc—dithizone complexes form two dithizone molecules, due to which the number of double bonds increases 2-fold in comparison with the 1:1 complex [1].

Hence, our study has shown that intravenous injection of cysteine in a dose of 1000 mg/kg to rabbits prevents the formation in β cells of complexes with zinc in response to subsequent injection of diabetogenic zinc-binding substances, causing cell destruction and development of diabetes. Injection of cysteine to animals is associated with a sharply negative reaction to zinc in β cells, this indicating blocking of metal ions in them. Injection of cysteine in a dose of 1000 mg/kg several minutes after dithizone and formation of the zinc—dithizone complex has led to elimination of dithizone from the complex and prevention of diabetes development in the majority of animals. The probable mechanism of the preventive effect of cysteine is formation of 2:1 complex in β cells with fixation of zinc atom between SH group sulfur atoms of two cysteine molecules.

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TABLE 2. Content of Insulin, Zinc, and Zinc—Dithizone Complex Levels in β Cells (rel. units; $M \pm \sigma$)

Experimental conditions	Staining				
	aldehyde fuchsin (insulin)	zinc—dithizone complex	diethylpseudoisocyanine (insulin)	TSQ (zinc)	dimethylnaphthylmethane (Victoria 4R) (insulin)
Intact animals	1.00±0.08 (*n*=24)	—	1.00±0.08 (*n*=14)	1.00±0.08 (*n*=19)	1.00±0.12 (*n*=22)
Cysteine	—	—	—	0.12±0.03** (*n*=19)	
Dithizone	0.31±0.04* (*n*=26)	1.00±0.08 (*n*=18)	0.26±0.04** (*n*=26)	—	0.45±0.09* (*n*=22)
Cysteine+dithizone	0.84±0.06 (*n*=18)	0.16±0.02** (*n*=25)	0.93±0.09 (*n*=22)	—	—

Note. *$p<0.01$, **$p<0.001$ in comparison with the corresponding values in intact animals.

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