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Abstract

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KEYWORDS: ferric nitrilotriacetate, glucose metabolism, pancreatic islet cells, pancreatic islet zinc

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DEGRANULATION EFFECT OF FERRIC NITRILOTRIACETATE (Fe^{3+} -NTA) ON THE PANCREATIC ISLET β -CELLS: ITS ACUTE TOXIC EFFECT ON GLUCOSE METABOLISM

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Abstract. A single injection of ferric nitrilotriacetate (Fe^{3+} -NTA) caused a transitory increase in plasma immunoreactive insulin (IRI) and plasma immunoreactive glucagon (IRG) in rats. They reached maximum levels at 2 days after injection and returned to the normal range at 10 days. At 2 days after Fe^{3+} -NTA injection, blood glucose level was normal but the glucose tolerance test (GTT) was impaired. There was a further increase in plasma IRI level and IRG level was suppressed after glucose loading. At 10 days after Fe^{3+} -NTA injection, glucose tolerance was normal and IRI also returned to the normal range. No degenerative changes were found on H.E.-stained rat pancreatic tissue sections after Fe^{3+} -NTA injection. Histochemical staining, however, showed a reduction in β -granules and heavy metals (Timm's granules) from islet cells in the central area of the rat pancreatic islet 1 to 3 days after injection of Fe^{3+} -NTA. The fading remained in some islets even at 10 days after injection, but by then the β -granule distribution was restored in most islet cells.

The results indicate a single Fe^{3+} -NTA injection induced transitory instability of the pancreatic islet β -cell granules and the glucose intolerance with a hyperresponse of IRI.

Key words : ferric nitrilotriacetate, glucose metabolism, pancreatic islet cells, pancreatic islet zinc.

We have previously reported the induction of a diabetic state (hyperglycemia, glycosuria, ketonemia and ketonuria) in rats and rabbits after a daily injection of ferric nitrilotriacetate (Fe^{3+} -NTA) for two months (1, 2). Repeated blood withdrawals from these diabetic animals resulted in recovery from the diabetic symptoms. The iron granules of the liver and pancreas disappeared, and the β -granules were restored to control levels. In the present study, a single injection

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of Fe^{3+} -NTA was used, to see its effect on blood glucose, corticosteroid, immuno-reactive insulin (IRI) and immunoreactive glucagon (IRG) levels and the histological response of the pancreas to it.

MATERIALS AND METHODS

Experimental groups. A total of 152 inbred Wistar adult male rats (170-250 g body weight) and 18 albino adult rabbits (2,000-2,500 g) were used.

The rats were divided into six experimental groups. Group I rats ($N = 66$) were used for measurement of blood glucose, corticosteroid, IRI and IRG. They were divided into seven subgroups (I-a to I-g) of eight animals each. Groups I-a to I-f received a single injection of Fe^{3+} -NTA intraperitoneally at 7.5 mg Fe/kg body weight, and were killed at different time periods after injection. The animals of Group I-g were untreated.

The rats were killed as a group by blood-letting at the following times after Fe^{3+} -NTA injection: 3 h (Group I-a); 6 h (Group I-b); 12 h (Group I-c); 24 h (Group I-d); 48 h (Group I-e); and 10 days (Group I-f). Animals of Group I-g were killed with those of Group I-f. Rats in Group I-a to I-e were starved (water *ad libitum*) during the course of the experiment (1-48 h).

Upon sacrifice 6 ml of blood were taken from the orbital sinus of each animal and heparinized. The fresh blood was cooled with ice, and plasma was obtained by centrifugation. A part of the fresh plasma was used for glucose measurement. The remainder was kept at -20°C and used for estimation of corticosteroid, IRI and IRG. The pancreas was removed for histological and histochemical study.

Group II ($N = 36$) rats were used for the blood glucose tolerance test (GTT) and for determination of IRI and IRG levels. The animals were divided into six subgroups of six animals each. Group II-a animals were tested 2 days after a single injection of Fe^{3+} -NTA; Group II-b animals were tested at 10 days; and Group II-c animals were tested at 21 days. Group II-d animals were not treated (controls).

The other two groups received a single injection of an equivalent amount of colloidal iron (Blutal, Dainippon Pharmaceutical, Osaka), and were examined for GTT, IRI and IRG at 2 days (Group II-e) and at 10 days (Group II-f) after injection.

Group III-a rats ($N = 6$) received a single injection of Fe^{3+} -NTA, and an insulin tolerance test (ITT) was conducted 2 days later. Animals in Group III-b ($N = 6$) were not treated (controls).

Group IV, V, and VI rats were used for histochemical studies of the pancreas. Animals in Group IV ($N = 23$) received a single injection of Fe^{3+} -NTA and those in Group V ($N = 15$), an equivalent amount of colloidal iron. Group VI ($N = 3$) was the untreated control. The animals were killed by decapitation at different time intervals after iron injection; Group IV-a and V-a, 12 h after injection; Group IV-b and V-b, 2 days; Group IV-c and V-c, 3 days; IV-d and V-d, 7 days; and IV-e and V-e, 10 days. Pancreatic tissue preparations were made and examined histologically and histochemically as in Group I.

Rabbits were used for histochemical studies of zinc in the pancreatic islets. There were seven subgroups of two rabbits each. Group A, B, and D received Fe^{3+} -NTA i.p. once at 7.5 mg Fe/kg body weight per animal. They were killed at 1 day (Group A), 2 days (Group B) and 10 days (Group C) after injection by severing the carotid artery. Animals of Group D and E received a single colloidal iron injection in an amount equivalent to that of Fe^{3+} -NTA, and were killed at 1 day (Group D) and 2 days (Group E) after injection. Animals in Group F were untreated, and those in Group G received only NTA injection.

Preparation of Fe^{3+} -NTA solution. Iron stock solution (0.1 M) was made by dissolving ferric nitrate in 0.5 N HCl. Na_2 -NTA solution was made by dissolving 1.128 g of disodium nitrilotriacetate (Na_2 -NTA) (Eastman Kodak) into 70 ml of distilled water by magnetic stirring for 3-5 minutes. The Na_2 -NTA solution (68.5 mM) was added into 16 ml of stock iron solution. The pH of this mixture was raised to 7.0 by adding sodium bicarbonate powder under magnetic stirring; then distilled water was added to make total volume to 179 ml. This final Fe^{3+} -NTA solution, which contained 0.5 mg iron per milliliter, was kept in a brown bottle with a rubber stopper to prevent a rise in pH by losing CO_2 (2-4) (if the pH became higher than 8.0, it was readjusted to 7.0-7.4 by blowing CO_2 gas into the solution).

Biochemical measurements. The colloidal iron was ferric hydroxide chondroitin sulfate colloid (5) (Blutal, Dainippon Pharmaceutical, Osaka, Japan). Glucose concentration in plasma was estimated by enzymatic analysis (6), plasma corticosterone by fluorescence technique (7), and plasma IRI concentration by radioimmunoassay with Riakit (Dainabot Radioisotope Institute, Tokyo). Glucagon concentration in plasma was measured by radioimmunoassay with antiglucagon sera prepared with the C-terminal fragment of rat pancreatic IRG (8) (Otsuka Assay Laboratory, Tokushima, Japan).

Histological preparation. The pancreatic tissue was divided into four sections. One part was fixed with 10 % formalin in phosphate buffered saline (pH 7.3) for hematoxylin and eosin staining and for Perls' iron staining (9). Another part was fixed with pure ethanol for Okamoto's zinc detection test (10). Still another part was fixed with Bouin solution for staining of the pancreatic islet β -granules by Gomori aldehyde fuchsin staining (11). Some tissues were also fixed with hydrosulfide-saturated ethanol for Timm's heavy metal stain (12-14). The latter method is extremely sensitive but not specific for any heavy metal.

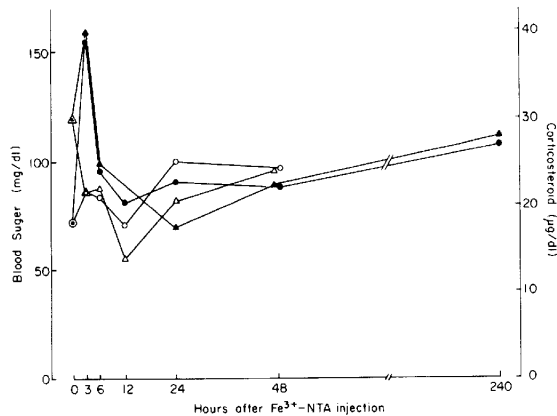
RESULTS

At 3 h after Fe^{3+} -NTA injection, the plasma glucose and corticosteroid concentrations were very high, but they dropped rapidly, reaching nearly normal levels at 6 h after injection (Text-Fig. 1). After the initial reaction their concentrations were stable for 10 days after injection.

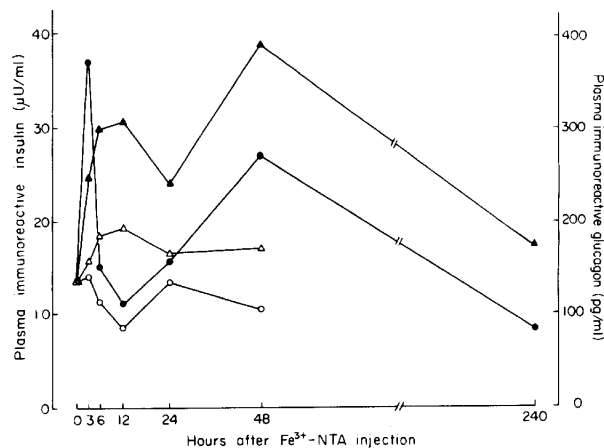
Plasma IRI also showed an early spike at 3 h after Fe^{3+} -NTA injection and dropped to a normal level at 6 to 12 h (Text-Fig. 2). At about 12 h, plasma IRI level began to rise, reaching its maximum at 48 h. The IRG level also showed similar second rise parallel to the change in IRI level. At 10 days after Fe^{3+} -NTA injection, both the IRI and IRG concentrations dropped to the normal ranges.

Oral glucose loading (1.2 g/kg body weight) conducted at 2 days (Group II-a), 10 days (Group II-b) and 21 days (Group II-c) after a single injection of Fe^{3+} -NTA or colloidal iron (Group II-e and II-f) produced hyperglycemia in all groups (Text-Fig. 3). The highest glucose response and a retarded recovery was found in animals tested 2 days after Fe^{3+} -NTA injection (Group II-a, Text-Fig. 3). The response was near the control level at 10 days after injection. Animals injected colloidal iron showed a similar reaction to the untreated controls.

Plasma IRI level was slightly high in rats tested 2 days after Fe^{3+} -NTA injection (Text-Fig. 4). Glucose loading at this point resulted in marked IRI elevation at 1 and 2 h (Group II-a, Text-Fig. 4). The hyperresponse of IRI was not ob-



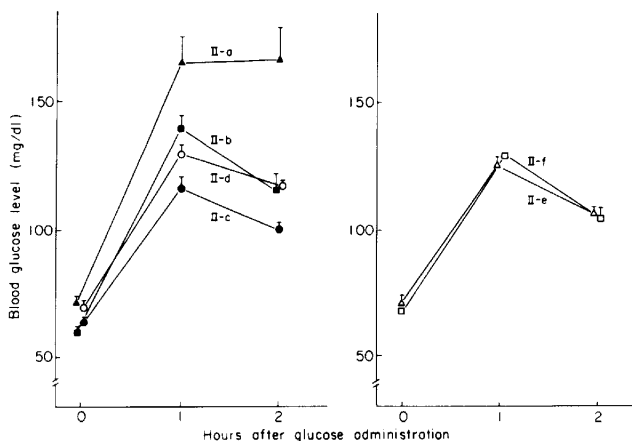
Text-Fig. 1. Plasma glucose and plasma corticosteroid concentrations after a single intraperitoneal injection of Fe^{3+} -NTA (7.5 mg Fe/kg body weight). Closed circles, plasma glucose concentration of Fe^{3+} -NTA-injected animals. Open circles, plasma glucose concentration of untreated controls. Closed triangles, plasma corticosteroid concentration of Fe^{3+} -NTA-injected rats. Open triangles, plasma corticosteroid concentration of untreated controls. Each point represents the mean of 8 animals.



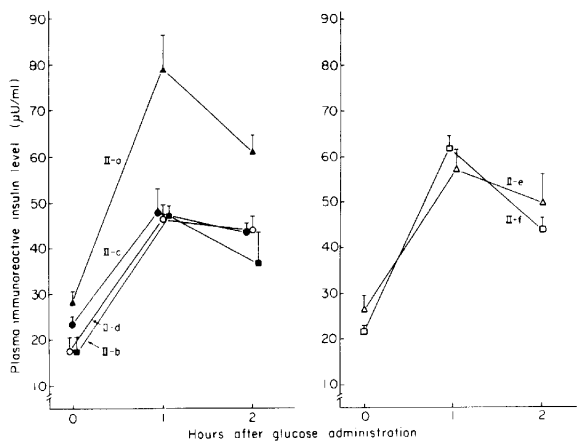
Text-Fig. 2. Plasma immunoreactive insulin (IRI) and plasma immunoreactive glucagon (IRG) concentrations in the same rats used in the Text-Fig. 1 data. Closed circles, plasma IRI concentration of Fe^{3+} -NTA-injected animals. Open circles, plasma IRI concentration of untreated controls. Closed triangles, plasma IRG concentration of Fe^{3+} -NTA-injected animals. Open triangles, plasma IRG concentration of untreated controls.

served in rats tested 10 and 21 days after Fe^{3+} -NTA injection. Animals previously received a colloidal iron injection (Group II-e and Group II-f) showed moderate elevations after glucose loading as compared to the untreated controls.

Plasma IRG level was also high in rats tested 2 days after Fe^{3+} -NTA injection (Text-Fig. 5). Glucose loading at this point induced a marked reduction in plasma IRG at 1 h (Group II-a, Text-Fig. 5). However, the IRG level was still higher

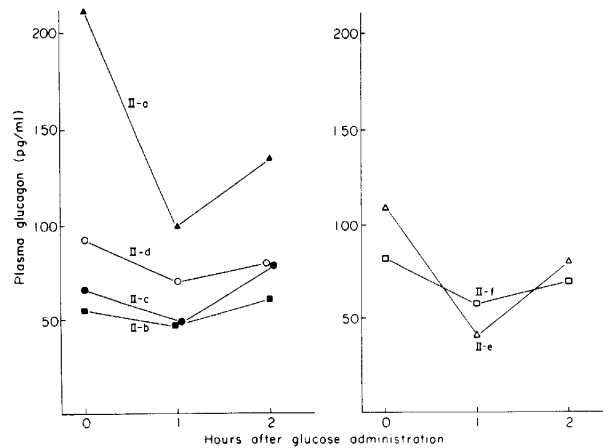
Degranulation Effect of Fe^{3+} -NTA on Pancreatic Islets

Text-Fig. 3. Glucose tolerance tests (GTT) of rats after injection of Fe^{3+} -NTA or colloidal iron. The oral loading dose of glucose was 1.2 g/kg body weight. II-a, Rats tested 2 days after receiving a single injection of Fe^{3+} -NTA. II-b, Rats tested 10 days after receiving a single injection of Fe^{3+} -NTA. II-c, Rats tested 21 days after an injection of Fe^{3+} -NTA. II-d, Untreated control rats. II-e, Rats tested 2 days after a single injection of an equivalent amount of colloidal iron. II-f, Rats tested 10 days after a single injection of an equivalent amount of colloidal iron. Each point represents the mean of 6 animals. The vertical lines represent standard deviations.

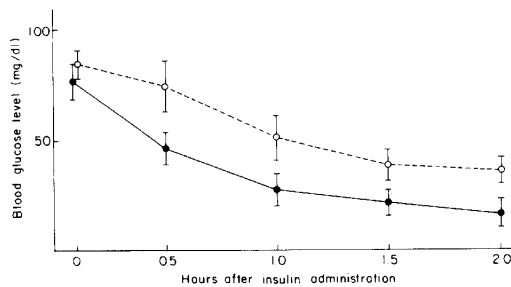


Text-Fig. 4. IRI levels in GTT of the same rats used for the Text-Fig. 3 data. The abbreviations in the graph are the same as in Text-Fig. 3.

than controls, and a further elevation followed 2 h after the glucose loading. At 10 days (Group II-b) and 21 days (Group II-c) after Fe^{3+} -NTA injection, the IRI level dropped slightly 1 h after glucose loading but recovered at 2 h. In rats previously received a colloidal iron injection, the IRI level was somewhat higher than the control at 2 days after injection (Group II-e) but glucose loading caused



Text-Fig. 5. IRG levels in GTT of the same rats used for the Text-Fig. 3 data. The abbreviations in the graph are the same as in Text-Fig. 3. The points represent the IRG values of pooled sera of equal quantities from six rats.



Text-Fig. 6. Insulin tolerance test (ITT) of rats injected with Fe³⁺-NTA. Insulin was injected at 0.2 U/kg. Closed circles, ITT of rats tested 2 days after a single injection of Fe³⁺-NTA (N = 8). Open circles, ITT of untreated controls (N = 8).

a reduction of IRG level at 1 h, as in the Fe³⁺-NTA injected group (Group II-a), and the IRG recovered to the control level. At 10 days after colloidal iron injection, the plasma IRG level was nearly normal and reacted like the control to glucose loading (Group II-f).

In ITT, blood glucose level decreased both in untreated control animals and animals tested 2 days after Fe³⁺-NTA injection (Text-Fig. 6). The extent of the decrease was much greater in the treated animals.

The H.E.-stained rat pancreas preparations at 12 h to 10 days after Fe³⁺-NTA injection revealed no distinct morphologic change. However, sections stained by Timm's reaction showed sequential morphological changes after Fe³⁺-NTA injection. In the untreated control, all islets appeared black and strongly stained, probably representing zinc. Silver granules are absent from the exocrine glands

(Fig. 1). At 12 h after injection, a slightly increased stainability in the islet was observed together with distinct brown-black silver particles in the exocrine glands which were not demonstrated by the conventional iron staining (Fig. 2). At 24 h the density of the silver granules was somewhat reduced, *i.e.* there was some fading of the silver staining in the central area, whereas it remained dark in the outlining areas of the islets. The exocrine glands had fine black granules inside the acinus. At 3 days after Fe^{3+} -NTA injection, the fading of the silver granules in the central area of the islet became distinct, with darkly stained cells occupying the outer areas of the islets showing heavy silver grains. Exocrine glands showed only fine black granules (Fig. 3). At 10 days after Fe^{3+} -NTA injection, a few islets still showed moderate fading of silver from the central area surrounded by dark outer areas (Fig. 4-a), but most islets had a nearly normal distribution of silver granules (Fig. 4-b).

Gomori aldehyde-fuchsin Masson staining of the healthy rat pancreas showed that β -granules were densely distributed in areas surrounding the vascular spaces (so-called polarization of β -granules) (Fig. 5). At 2 days after Fe^{3+} -NTA injection, the β -granules showed a decreased density, with compression of the vascular spaces probably due to edema; although the polarization structure of the β -granules towards the vascular spaces still remained (Fig. 6a, 6b). At 10 days after Fe^{3+} -NTA injection, the density of the islet β -granules almost recovered, and the polarization pattern became distinct in some lobes (Fig. 7), but in other lobes the islet still showed the fading of granules.

Animals receiving a colloidal iron injection showed less fading of the Gomori β -granules in the inner islet area at 24 to 48 h after iron injection (Fig. 8a, 8b). Ten days after colloidal iron injection, there was a complete recovery of β -granule distribution, and the granules were polarized towards the vascular lumen (Fig. 9).

Timm's silver stainings of rabbit pancreatic tissues did not show any significant difference between Fe^{3+} -NTA injected and untreated animals. Both showed dense darkly stained pancreatic islet cells (Figs. 10-13); these were stained much darker than the islets of untreated rats (Fig. 1). Exocrine glands of control animals had no silver granules (Fig. 10). At 1 and 2 days after Fe^{3+} -NTA injection, some aggregated fine granules were seen within the acinus (Figs. 11, 12). They cannot be recognized by the conventional iron staining because of the insufficient sensitivity of the method. Some fine granules were found in exocrine glands even at 10 days after iron injection (Fig. 13).

Gomori aldehyde-fuchsin staining of the pancreas of untreated rabbits (Fig. 14) and NTA-injected controls gave results which were nearly the same as that of untreated rats. At one day after Fe^{3+} -NTA injection, rabbit β -granules were faded and the vascular spaces were compressed with edematous cell swellings, though some granule polarization still remained (Fig. 15). At 2 days after Fe^{3+} -NTA injection, the fading of β -granules became marked, and the polarization of

the granules was nearly lost (Fig. 16). At 10 days, the density of the β -granules recovered and was denser in some lobes than in the control pancreas (Fig. 17b). The polarized pattern was generally restored, though some moderate fading of β -granules was still observed with disordered polarization (Fig. 17a).

Zinc staining of the normal rabbit pancreas (Okamoto's staining) gave fine granules with bright reddish-purple tint to islet cells (Fig. 18). Fe^{3+} -NTA injection resulted in : fading of the islet cell stain, a marked decrease in color reaction one day after injection (Fig. 19) and an almost complete loss of reaction at 2 days after injection (Fig. 20). At 10 days after Fe^{3+} -NTA injection, the color reaction appeared nearly normal with a clear reddish-purple zinc color (Fig. 21), though some islets showed a faint reaction.

Colloidal iron injection also induced a slight fading of the zinc reaction, but it was less compared to that in Fe^{3+} -NTA injected animals (Figs. 22, 23).

DISCUSSION

An intraperitoneal injection of Fe^{3+} -NTA induced a marked transitory hyperglycemic reaction at 3 h after injection. A concomitant increase was found in plasma corticosteroid level, suggesting generalized reactions to tissue injury probably caused by free radicals formed 1 and 3 h after Fe^{3+} -NTA injection (15). Two days after Fe^{3+} -NTA injection, a high level of plasma insulin was found, even though the animals were starved during this period and the plasma glucose level remained in the normal range. This was temporally associated with the fading of silver granules in Timm's staining or the decrease of β -granules in Gomori's staining in the pancreatic islet cells. No suitable explanation for the precipitating factor for the delayed massive release of insulin caused by Fe^{3+} -NTA is available at present. The increased plasma level of glucagon may be associated with the release of insulin or related to the hepatic injury induced by Fe^{3+} -NTA (16). At this stage, the glucose tolerance was impaired in spite of the hyper-response of insulin secretion. This resembles the glucose intolerance in hepatic injury (17), although the insulin resistance was not demonstrated in insulin tolerance test in this study. High IRG level was suppressed in glucose tolerance test, indicating that the glucagon secretion is operating normally under the conditions of impaired glucose tolerance in Fe^{3+} -NTA-treated rats. The acid ethanol extract from the rat plasma at this stage of Fe^{3+} -NTA treatment was compared with that of the untreated controls after glucose loading. The extract was loaded on a Biogel P-30 column and the eluted fractions were measured by radioimmunoassay. The plasma IRI of Fe^{3+} -NTA-injected rats was found to be insulin, and not proinsulin (unpublished data). The injection of colloidal ferric iron did not induce such a severe functional disturbance of the pancreatic islet. Thus, the ferric iron in the form of Fe^{3+} -NTA produces free radicals (15) *in vivo*.

In the previous study of long term Fe^{3+} -NTA administration, iron was diffusely deposited in pancreatic islet cells (1, 2). The mechanism causing the re-

lease of insulin by Fe^{3+} -NTA might be the exchange in the islet of zinc and ferric irons, making the condensed insulin molecule unstable. Timm's staining of the rat pancreas indicated an depletion of heavy metal from the central area of the islet at 2 to 3 days after Fe^{3+} -NTA-treated rat in Zn is supported by demonstration of Zn depletion in rabbit pancreatic islets by Fe^{3+} -NTA treatment. Since rabbit islet cells contain ten times more Zn as compared with rat islet cells, it was possible to directly visualize Zn by a histochemical method of Okamoto. Keeping in line with this is the finding that the islet areas with scanty Timm's reaction in rats contained mainly β -cells and the density of the secretory granules of β -cells was reduced in the corresponding areas. Therefore, it may be inferred that zinc is lost from β -cells in Fe^{3+} -NTA treatment. The increased plasma insulin level in 2 days of Fe^{3+} -NTA treatment is not due to simple destruction of β -cells, because the cells did not show any degenerative changes. At 10 days after Fe^{3+} -NTA injection, when Gomori's stain indicated a recovery, β -granules were dense again and blood insulin level was normal. The *in vitro* degranulation effect of Fe^{3+} -NTA has been observed in the tissue culture level using the young rat pancreatic islet cells (18, 19).

Several lines of evidence have indicated that zinc plays an important role in insulin production and microcrystalline formation in the insulin granule (20-23). It is well-known that agents having an affinity to zinc, such as alloxan and dithizon, induce diabetes in animals (24, 25). The accumulated *in vivo* and *in vitro* evidence suggests the competition of zinc and iron for the ferritin molecule (26-28). Therefore, the ferric ions in Fe^{3+} -NTA may expel zinc from β -cells, make insuline labile and allow its release from β -cells. The concomitant increase in plasma glucagon indicates that Fe^{3+} -NTA also unstabilize the α -cell granules. The cell may also have zinc in it. Grimelius' staining of rat pancreatic α -cells showed a transient reduction of α -cell granules at 2 days after Fe^{3+} -NTA injection (29).

It has been revealed that alloxan and other diabetogenic chemical agents inhibit the function of the membrane superoxide dismutase (SOD) of pancreatic islet cells by generating the superoxide and hydroxyl radical (30, 31). This state may have been also induced by Fe^{3+} -NTA because the introduced iron enhances the generation of the hydroxyl radical (32, 33). The alteration of cell membrane permeability might act on islet cells to stimulate the liberation of α - and β -granules into the blood circulation. Thus, the expelling of zinc by ferric iron seems to be the most plausible mechanism of the Fe^{3+} -NTA intoxication. Repeated injections of Fe^{3+} -NTA may have induced a constant release of islet granules into peripheral circulation, which forces islet cells to synthesize new granules, and this eventually may exhaust the islet cells. This would explain the diabetic signs observed in our animals after repeated Fe^{3+} -NTA injection (1, 2).

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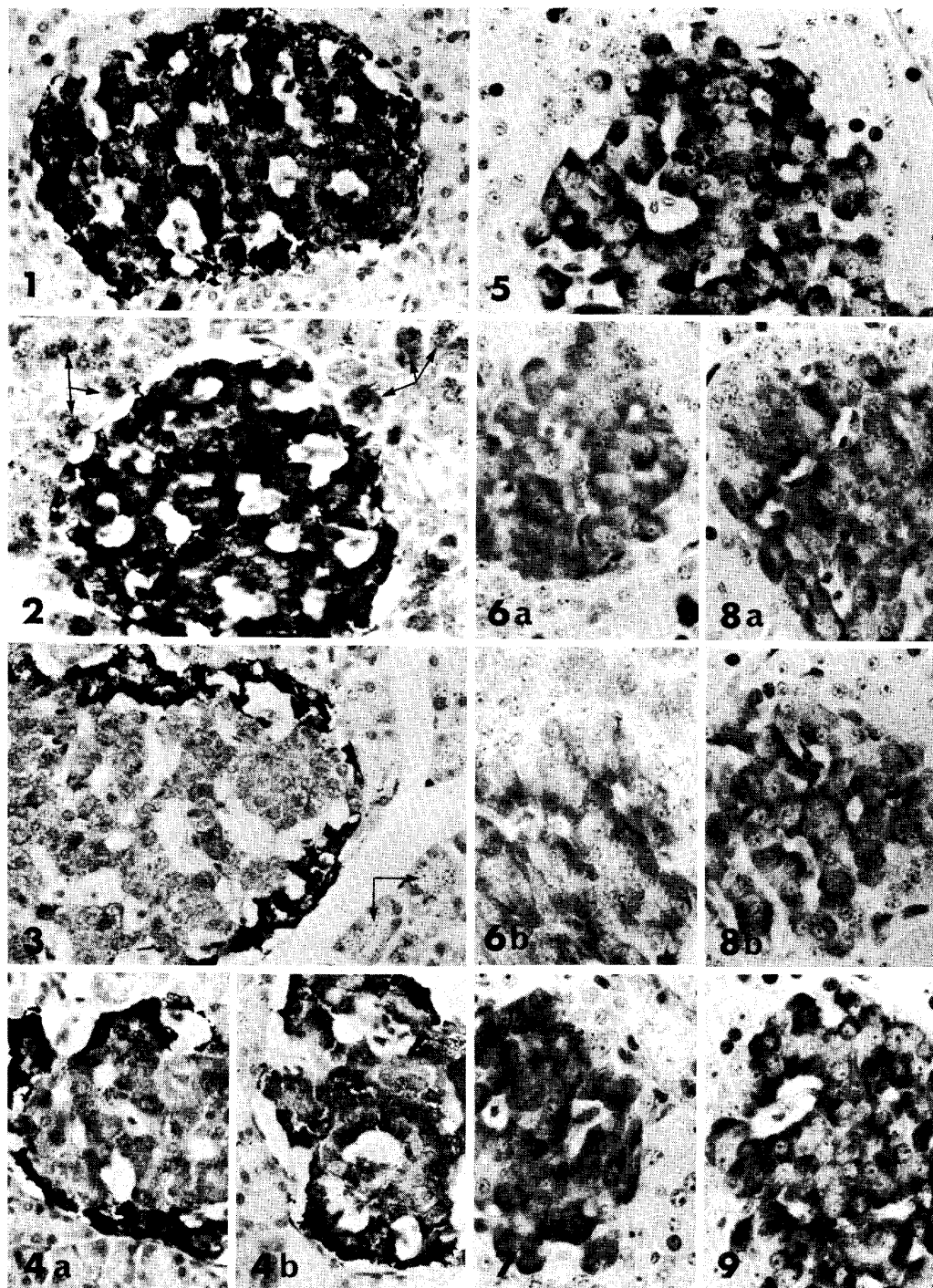
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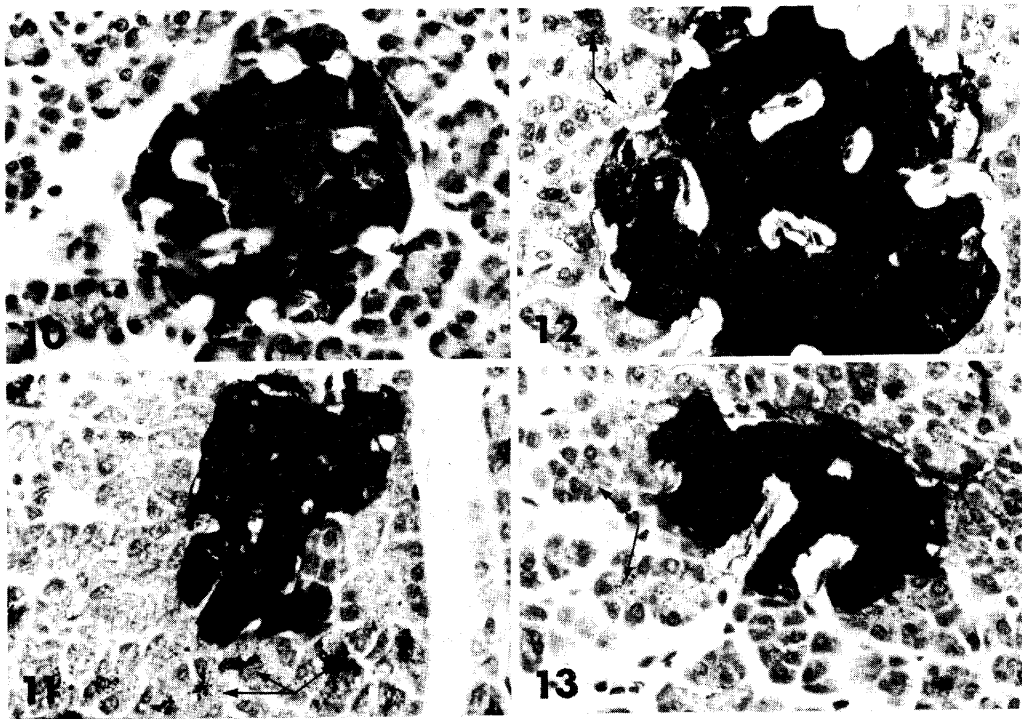
Figs 1 to 4 show rat pancreatic islet cells stained by Timm's silver staining for heavy metals ($\times 300$).

Fig. 1. Untreated control. All islet cells appear black and are strongly stained, probably representing zinc. Silver granules are absent from the exocrine glands. Fig. 2. Twelve h after Fe^{3+} -NTA injection. The islet cells are stained black as the controls, but exocrine cells have black-brown silver particles in areas facing the acinar lumen (shown by arrows), due probably to excreted iron. They cannot be recognized by the conventional iron staining probably due to an insufficient sensitivity of the method. Fig. 3. Three days after Fe^{3+} -NTA injection. Most islet cells appear gray, surrounded by cells stained black. The fading of the silver granules indicates the escape of heavy metals, probably zinc. Fine silver granules deposited in exocrine gland cells along the acinar lumen (shown by arrows) probably indicate iron excretion. They are so small in amount, therefore cannot be recognized by the conventional iron staining. Fig. 4. Ten days after Fe^{3+} -NTA injection. Some islets had cells with moderate silver fading (Fig. 4a), whereas others had dense silver granules, indicating the restoration of zinc (Fig. 4b).

Figs. 5 to 9 show rat pancreatic islet cells stained by Gomori aldehyde-fuchsin stain ($\times 300$).

Fig. 5. Untreated control rat. Beta cells show abundant granules with a polarized distribution toward the vascular lumen. Figs. 6a, b. Two days after Fe^{3+} -NTA injection. There is a reduction of β -granule density. Fig. 7. Ten days after Fe^{3+} -NTA injection. The islet shows a recovery of β -granules. Fig. 8a, b. Two days after colloidal iron injection. The islet shows a slight decrease of β -granules. Fig. 9. Ten days after colloidal iron injection. There is a complete recovery of β -granule distribution, and the granules were polarized towards the vascular lumen.





Figs. 10 to 13 show rabbit pancreatic islet cells stained by Timm's silver staining ($\times 300$).

Fig. 10. Untreated control. The pancreatic islet is stained darker than in the untreated rat pancreatic islet (Fig. 1) because of a higher concentration of zinc in the rabbit islets. Exocrine glands have no silver grains. Figs. 11 and 12. At 1 and 2 days after Fe^{3+} -NTA injection. The exocrine glands have silver granules distributed roughly along the acinar lumen (shown by arrows), which probably show iron secretion though they are not demonstrated by the conventional iron staining method. Fig. 13. Ten days after Fe^{3+} -NTA injection. The islet cells are stained homogeneously and densely. The exocrine glands have finer silver granules, with a similar distribution pattern to Figs. 11 and 12 (shown by arrows).

Figs. 14 to 17 show rabbit pancreatic islets stained by Gomori aldehyde-fuchsin stain ($\times 300$).

Fig. 14. Untreated control. The beta cell granules are polarized towards the vascular space. Figs. 15 and 16. At 1 and 2 days after Fe^{3+} -NTA injection. Beta cell granules show a lower density, though their distribution is still somewhat polarized. Fig. 17. Ten days after Fe^{3+} -NTA injection. Some cells still show a decreased granule density (Fig. 17a). Fig. 17b shows most lobes with a nearly normal β -granule density.

Figs. 18 to 23 show rabbit pancreatic islet cells stained by Okamoto's zinc staining method.

Fig. 18. Untreated control. Islet cells show densely distributed fine granules of zinc stained reddish-purple (they appear as dark granules in the micrograph). Figs. 19 and 20. At 1 and 2 days after Fe^{3+} -NTA injection. Both islet cells show a nearly complete loss of zinc granules. Fig. 21. Ten days after Fe^{3+} -NTA injection. The reddish-purple zinc color reappears in most of the lobes. Figs. 22 and 23. At 1 and 2 days after colloidal iron injection. A slight loss in zinc intensity is evident but the loss is less than in the Fe^{3+} -NTA injected animals (Figs. 19 and 20).

