Production of Experimental Diabetes Mellitus and Zinc Reaction of Islets of Langerhans

OKAMOTO, Kozo

(Citation)
The Kobe journal of the medical sciences,1(1):77-88

(Issue Date)
1951

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100489366

Production of Experimental Diabetes Mellitus and Zinc Reaction of Islets of Langerhans.

Kozo OKAMOTO,

with Collaboration

of

Z. AOYAMA, T. IBARAKI, H. NARUMI, M. SHIBATA, Y. KAWASAKI, D. SHIBATA and K. KOMATSU.

(Department of Pathology, Hyogo Prefectural Medical College, Kobe.)

I. Introduction

Nine years ago, one of the present authors, Okamoto (1) reported a new histochemical test method of zinc in tissues. And applying this method, he observed that the islets of Langerhans contain an appreciable amount of this metal (1,2), and it occurred to him that one of the important biological meaning of zinc in the islets might be its pathological significance closely related to the production or the progress of diabetes mellitus. With collaboration of I. Kadota (3,4), he performed a series of experimental research and found that, except alloxan, a well-known diabetogenic reagent (5,6,7,8) having an affinity to zinc, both oxine and dithizone, which are also effective reagent to combine with zinc, have a power of producing diabetes mellitus on animals. The results let them to a conclusion that diabetes mellitus might be caused by administration to animals of a zinc-affine reagent which is injurious to tissue cells, as the reagent combines with the cells of the islets of Langerhans does harm to the function of the cells. Then Okamoto came to an anticipation that, when a reagent, which is zinc-affine but uninjured to tissue cells, is administered, it may combine with the cells of the islets; not only does it harmless to the cells, but also it may prevent the cells from being combined with other diabetogenic reagents of zinc-affine nature, so that diabetes mellitus might not be occurred. This anticipation was proved to be true by experiments of D. Shibata and T. Ibaraki, performed with the suggestion of Okamoto, that the two zinc-affine reagents of non-injurious character, anthranilic acid and quinaldinic acid, have a preventive action against production of experimental diabetes by alloxan, oxine or dithizone. The results were referred briefly in Okamoto's paper (3), of which the detail will soon be published elsewhere.

Now next anticipation flashes on them that the degree of production of diabetes mellitus by those zinc-affine diabetogenic reagents may intimately be connected with the quantity of zinc in the islets cells; that is, the more the islet cells contain zinc,

the more amount of the zinc-affine diabetogenic reagent will combine with the islet cells and may cause severe damages to them, and an advanced diabetes mellitus may be followed. Present research was undertaken to answer the question if this anticipation be true or not.

II. Method and Materials

(1) Animal:

Dog, cat, rat, mouse, guinea pig, adult rabbit, healthy pregnant rabbit as well as infant rabbit were used. Before the administration of the reagents these animals were kept away for 10 days or more from food, which contains the abundance of vitamin B.

(2) Diabetogenic reagent:

(a) Alloxon monohydrate (Merck) was used as 1 to 5 per cent solution in distilled water (5,6,7,8).

(b) Oxine (supplied by Japanese pharmacist, Takeda or Hayashi) was first dissolved in dilute hydrochloric acid and then was prepared to make 0.5 to 2 per cent solution with distilled water (3,4).

(c) Dithizone (Takeda, Merck etc.).
First 100 to 200 mg. of the reagent was added to 10 cc. of 0.2—0.5 per cent ammoniac solution, previously warmed to 70°C, and the mixture was warmed for 10 minutes at the same temperature and filtered after being cooled and soon the filtrate was used (3,4).

All these diabetogenic reagents were injected intravenously. According to the kind of animals, veins of ears, paws or tails were adapted for this purpose.

(3) Hormone:

Prae-hormon (anterior pituitary hormone), Thyradin (thyroid hormone) and Interenin (adrenal cortical hormone) were used by means of subcutaneous injection: 1 cc. of thyradin corresponded to 0.1 gm. of dried thyroid; 1 cc. of interenin to 0.1 gm. of fresh adrenal cortex of cattle.

(4) Examination:

(a) Urine sugur was estimated by Benedict's method.
Judged as follows, after adding the urine 6 drops to the reagent 3 cc. and 2 minutes boiling:
Bluish-green solution without the precipitation is ±. (The quantity of urinary sugar is less than 0.02%.)
Bluish-green solution with the yellowish-blue precipitation is +. (The quantity of urinary sugar is ca 0.02-0.2%.)

Bluish-green solution with the red-brown precipitation is ++. (The quantity of urinary sugar is ca 0.2-0.5%.)

Green solution with the reddish-brown precipitation is +++. (The quantity of urinary sugar is ca 0.5-1.5%.)

Non-colored solution with red precipitation is ++++. (The quantity of urinary sugar is more than 1.5%.)

(b) Blood sugar was determined by Hagedorn-Jensen's method.

(c) For histological examination the routine histological staining followed. Besides, islets of Langerhans were examined by Gomori's stain method (9), and the zinc test method by Okamoto and Hashimoto (10). Samples of pancreatic tissue were taken from 3 portions of the organ, i.e. one from the portion situated near duodenum, another near spleen, and the third from the middle of the mentioned two portions. These samples of the tissues were divided into small pieces, each of which was examined separately. In every case the numbers of α and β cells of 20 to 50 islets of Langerhans were measured, which was stained by the method of Gomori, and the average numbers of them were assumed to be the numbers of α and β cells of one islet.

Regard to the test method of zinc, at first Okamoto (11) reported diphenylthiocarbazide method in 1942, and later in 1944, Okamoto and Hashimoto (10) reported in detail about this method added with the dithizone method. According to several circumstances this method is not generally known and many inquiries have arose from various districts. Therefore, its main procedure will be described for the reference:

(A) Diphenylthiocarbazide method.

1. Fixation with absolute alcohol.
2. Make paraffin, celluloidin or celluloidin-paraffin sections.
   (Deparaffinization through xylol and absolute alcohol in paraffin or celluloidin-paraffin sections.)
3. Transfer the sections to the following mixture, for two or three hours or more at room temperature:

   Dilute ammonia water of pH 8.4-9 ..... 50 cc.
   *Solution of diphenylthiocarbazide saturated in 60 per cent alcohol .....1-3 cc.

   *The solution is to be used during two or four days after its preparation. (i.e. This solution is less suitable if used within two days or after four days of its preparation.)
4. Rinse the sections in water (running water may be used. Do not rinse for a long time); stain the nucleus with hematoxylin. (Do not differentiate in acid alcohol solution. The rinse is limited to 15 minutes). Mount in glycerin.

Findings :

The zinc granules are stained deep violet red (purple red). (vide Fig. 1, 3). Preservation of the preparation for a long time is impossible.

Caution :

1. In this test method, the section materials must be fresh, that is soon after animal's death.
2. The above-mentioned ammonia water may be replaced by a caustic soda and sodium acetate.
3. Prepare the reagents with pure distilled water. Redistilled water is preferable.

Differentiation :

Sometimes it requires differentiation from lead, mercury, bismuth, cadmium, cobalt and silver. (But usually they do not exist physiologically in animal tissues.) Differentiation between cobalt and zinc is easily recognized by the difference in the color of the reaction products between metals and reagent. Other metals can also be differentiated from zinc, exclusive of mercury (with the exception of corrosive sublimate or similar compounds), by fair difference in color of the reaction products.

Differentiate as follows, if necessary :

1. Put the sections in the following mixture for two or three hours or more at room temperature.

1 per cent aqueous solution of ammonium chloride which is prepared with its pH at about 8.4-9 with dilute ammonia water ..... 50 cc.
Solution of diphenylthiocarbazide saturated in 60 per cent alcohol as mentioned above ..... 1-3 cc.

The negative reaction shows zinc or cadmium, but not lead, mercury, bismuth or silver.

2. Then put the sections in the following mixture for two or three hours or more at room temperature.

1 per cent aqueous solution of ammonium carbonate which is prepared with its pH at about 8.4-9 by dilute ammonia water or acetic acid ..... 50 cc.
Solution of diphenylthiocarbazide saturated in 60 per cent alcohol as above mentioned ..... 1-3 cc.

Zinc gives no reaction, while cadmium reacts positive. (Lead and bismuth also react.)

(B) Dithizone method.

1. Make sections in the same manner as (A).

2. Put the sections into the following mixture for three hours or more at room temperature.

{Dilute ammonia water of pH 8.4-9 (or an aqueous solution of caustic alkali of pH 8.4-9) ..... 50 cc.
Dithizone solution saturated in alcohol (newly prepared) .....1-3 cc.

3. Rinse the sections in water, stain the nucleus with hematoxylin, mount in glycerin.

Findings:

The zinc granules are stained violet red (purple red).

Preservation of the preparation for a long time is impossible.

Differentiation:

In this method, mercury has a possibility of reacting similarly to zinc (with the exception of corrosive sublimate or similar compounds). Differentiation is to be done, if necessary, in the same manner as (A), using the mixture of ammonium chloride and ammonia water.

The dithizone method is inferior to that of diphenylthiocarbazide.

III. Results

1. Results on animals of different species.

In 1942 (1) and 1943 (2), Okamoto reported that the zinc content of the islet cells of Langerhans detected by means of his histochemical method varies according to the species of animals, their age and the conditions. In the present research it was observed that the administration of several hormones has a considerable influence on the zinc content of the islet cells.

On each five adults animals of several species, the zinc content of the islet cells were studied. The order according to the zinc content is observed as follows:

rabbit > dog > white rat, mouse > guinea pig

Red color of Fig. 1 indicates clearly a rich content of zinc in the islet cells of the rabbit. In dog, rat or mouse, the coloration is certainly lighter than that of the rabbit, while the difference between the former two is much less distinct. In guinea pig the islet cells show no coloration to zinc, though this does not mean that the cells of this animal contain no zinc, but it means that the zinc content is so poor that its histochemical detection is impossible. In case the islet cells are damaged by alloxan or other diabetogenic reagents, the zinc content of the cells decreases rapidly and after one or two days of administration the zinc reaction almost disappears. Fig. 2 indicate an intermediate state before disappearance of the zinc reaction.

On the other hand, diabetic states of different animals caused by the mentioned diabetogenic reagents are summarized in table 1.

Table 1.

The Degree of the Production as well as the Progress of Alloxan, Oxine and Dithizone Diabetes of each Kind of Animals

		Rabbit	Dog	Cat	Rat	Mouse	Guinea pig
Alloxan	Total number used	30	3		20	35	7
	Dose of administration	200 mg/kg (intravenous)	50 mg/kg (intravenous)		50 mg/kg (intravenous)	50 mg/kg (intravenous)	200 mg/kg (intravenous)
	Diabetes (more than one week duration)	19	3		14	15	1
	Diabetes (more than one month duration)	14	2		7	5	0
	Degree of diabetes	###	###		## ~ ###	## ~ ###	## ~ ###
Oxine	Total number used	18	6	5	40	35	20
	Dose of administration	50 ~ 60 mg/kg (intravenous)	50 ~ 70 mg/kg × 1~3 (intravenous)	30 mg/kg × 1~2 (intravenous)	60 ~ 100 mg/kg (intravenous) 100 mg/kg (intraperitoneal)	30 mg/kg × 1~4 (intravenous)	100~120 mg/kg (intravenous or intraperitoneal)
	Diabetes (more than one week duration)	10	3	4	4	3	5
	Diabetes (more than one month duration)	3	2	2	0	0	0
	Degree of diabetes	###	+ ~ ##	+ ~ ## (sometimes ###)	+ ~ ###	+ ~ ##	+ ~ ##
Dithizone	Total number used	42	4	3	40	25	8
	Dose of administration	100 mg/kg, 0.2% ammoniac solution (intravenous)	do	do	do	200 mg/kg, 0.2% ammoniac solution (intravenous)	100 mg/kg, 0.5% ammoniac solution (intravenous)
	Diabetes (more than one week duration)	33	4	0	5	2	2
	Diabetes (more than one month duration)	24	3	0	1	0	0
	Degree of diabetes	###	+ ~ ##	+ ~ ##	+ ~ ###	+ ~ ##	+ ~ ##
	Remarks			Glycosuria and no appetite for the first 3 or 4 days		Generally shows high mortality	

(1) The total number used does not coincide with the sum of the number of the diabetic animals of more than one week duration, and that of the animals of more than one month duration. The differences show those recovered or died during the period, some of those being sacrificed for histological examination.

(2) As the accurate dose of dithizone can not be indicated, the dose given shows concentration of ammoniac solution and the dose of reagent which is taken in 10cc. of its solution. Instead of ammoniac, lithium carbonate may be used.

(3) Data of oxine was obtained during a period of from June to October. In winter, production of oxine diabetes is no easy matter.

In this table it is shown that, when alloxan is administered, severe and permanent diabetes is produced most easily on rabbits or on dogs : in rats or mice diabetes produced experimentally is somewhat lighter, while in guinea pigs it occurs as rather slight and temporary. Results produced by oxine and dithizone coincide with each other in rabbits which suffer most severely, dogs and cats follow them ; while rats, mice and guinea pig suffer only slightly and temporarily. In evaluating the experiments it should be borne in mind, that the doses of reagents administered are not all the same ; but it is certain that experimental severe diabetes could easily be produced on rabbits : while the effect is slightest on guinea pigs, and other animals sitting between these two species. That the administration of alloxan can hardly produce diabetes on guinea pigs and cats is an already known fact (5-8,11,12).

Thus, it is confirmed that the rabbit, which has the richest content of zinc in the islet cells, is most easily be seized by experimental diabetes on administration of alloxan, oxine or dithizone, all of which being zinc-affine and at the same time injurious to the islet cells. While the guinea pig, which contains almost no detectable zinc in the islets cells is most resistant against occurrence of the experimental diabetes. Other experimental animals such as the dog, cat, rat or mouse place between the above mentioned two species, in their order of zinc content of the islet cells and on occurrence of experimental diabetes by the zinc-affine reagents.

2. Results as to the age of the rabbit.

In fetal life period and several periods after birth, the zinc content of the islet cells of rabbits were examined by the histochemical method and the following results were obtained :

Fetus of 20~28 days from pregnancy	New-born	15 days after birth	30 days after birth	60—100 days after birth	Adult
−	−	±	++	++	++

Thus, it will be seen that the detectable zinc of the islet cells begins to appear at first about 15 days after birth, and it increases gradually until it reaches to the adult level some 60-100 days after birth.

At the corresponding periods of the animal, effect of administering dithizone were examined. Table 2 gives the results.

Though the islets of the mother rabbit is severely damaged by administration of the reagent, there is seen no changes in the islets of its fetus, and blood sugar of the latter is kept at normal level 12 hours after birth and thereafter. It is also revealed that a slight injurious effect of dithizone is seen on the islets of infant rabbit of about 15 days after birth, though no sign of glycosuria is seen. With young rabbits

Table 2.

The Age of Rabbit and its Degree of Production of Diabetes Mellitus.

Age	Number	Data		Number of the cells of the islets of Langerhans (average number of one islet)		Remarks
		Blood sugar at the end of experiment (2 to 7 days after injection) (average mg/dl)	Urine sugar during of experiment	α-cell	β-cell	
Fetus (22 to 28 days after pregnancy)	6	Equal to mother rabbit soon after delivery, 80~110 (after 12~24 hours)		5.0	30.0	New-born after 1 to 8 days after the administration of reagent to mother rabbit. Sacrificed during from 6 hours to 3 days after delivery.
Mother rabbit of fetus mentioned above	4	Over 385	###	15.6	4.8	Sacrificed during 6 hours to 3 days after delivery.
3 days after delivery	1			7.5	26.8	
5 days	2	84	−	10.9	31.9	
13~16 days	4	141	−	10.0	28.9	Examined cases of the islet of Langerhans.....2
20~25 days	2	173	±	20.0	23.5	
30~36 days	7	203	+	19.3	17.8	
40 days	3	270	## ~ ###	8.1	3.7	Same above described.....1
50 days	2	307	###	9.6	4.7	Same above described.....1
Control, 3 to 50 days after delivery	7	86~121	−	9.7 (6.1~16.5)	32.5 (24.2~39.3)	Sacrificed as control properly from 3 to 50 days after delivery

Note :— (1) All fetus were injected dithizone, 10cc. per kg. (0.2 per cent ammoniac solution, 100mg. of dithizone) intravenously.

(2) Mother rabbit was first injected with alloxan intravenously, 75mg. per kg. and then dithizone, 10cc. per kg. after 5 to 7 days from the injection of alloxan.

(3) No hypoglycemic convulsions in infant rabbit were seen before 30 days after delivery occasionally convulsions were recognized after 30 days from delivery.

(4) It is a little difficult to differentiate α-cell and β-cell of the rabbit before 30 days after delivery. In this period the number of the cells of the islets of Langerhans of control seems to be fewer compared with adult rabbit. (α-cell 6.1 to 10.8, β-cell 24 to 30 in one islet of Langerhans.) (In adult rabbit, α-cell.....13, β-cell.....38.)

of 1 month of age, mild symptoms of diabetes were observed; the zinc content of its islets being more pronounced, while, with the animal of about 40 days after birth or thereafter, the diabetogenic reagent causes diabetic symptoms in the same intensity as on adult rabbits.

These results indicate clearly that the diabetic effect of the diabetogenic reagent goes parallel with increase in the zinc content of the islets of Langerhans of rabbits with lapse of days after birth.

Table 3.

The Administration of Hormone and the Production of Diabetes Mellitus.

		Administration of hormone (Administered before injection of diabetogenic reagent)			Dose of diabetogenic reagent (intravenous)	Number of animal	Duration of observation after injection of diabetogenic reagent (day)	Data		Finding of islet of Langerhans (average number of the cells of one islet of Langerhans)	
		Classification	Injected dose	Duration of administration (day)				Urine sugar (during experiment)	Blood sugar at the end of experiment (average mg/dl)	α-cell	β-cell
Rabbit	Normal					6		–	102	15.9	37.4
	Dithizone	Control			50mg/kg*	2	6	± ~ ±	268	18.4	17.6
		Praehormon	100 unit/kg subcutan. inj. 1 time 1 day	5	〃	2	6	±	373	13.2	7.2
		Thyradin	4 cc/kg 〃 〃	5	〃	2	6	±	412	14.7	8.8
		Interenin	1 cc/kg 〃 〃	5	〃	2	6	±	385	14.2	5.8
	Oxine	Control			50mg/kg	4	4 ~ 8	– ~ ±	170	19.2	42.9
		Praehormon	100 unit/kg subcutan. inj. 1 time 1 day	5	〃	2	4	± ~ ±	385	17.9	14.3
		Thyradin	4 cc/kg 〃 〃	5	〃	2	4	± ~ ±	251	22.3	11.6
		Interenin	1 cc/kg 〃 〃	5	〃	2	4	± ~ ±	304	31.6	15.7
Rat	Normal					5		–	104	16.0	62.5
	Alloxan	Control			50mg/kg△	4	4 ~ 7	– ~ ±	171	22.7	60.3
		Praehormon	100 unit/kg subcutan. inj. 1 time 1 day	5	〃	2	6	± ~ ±	337	29.6	27.1
		Thyradin	4 cc/kg 〃 〃	5	〃	2	6	± ~ ±	360	28.7	24.8
		Interenin	1 cc/kg 〃 〃	5	〃	2	6	± ~ ±	313	27.4	25.9

Note :— (1) Experimental animals were fed keeping away from the abundance of vitamine B before the experiment for more than 10 days.

(2) *Taken 100mg. of dithizone in 10cc. of 0.2 per cent ammoniac solution, warmed to 70°C for 10 minutes, injected 5cc. per kg. (a half dose of diabetogenic dose).

(3) △As alloxan was stale, its effect was a little, but comparison made with the each result as the same reagent injected at the same time.

(4) Experiment of oxine was performed in winter (room temperature 20°C).

(5) 1cc. of thyradin represented 0.1 gm. of dried thyroid.

(6) 1cc. of interenin represented 0.1 gm. of fresh adrenal cortex of cattle.

3. Influence of several hormones on the rabbit.

The following three hormones were chosen, each of which were given to two adult rabbits in the following way.

praehormon :	100 unit/kg,	once a day for	5 days successively
thyradin :	4 cc./kg,	,,	,,
interenin :	1 cc./kg,	,,	,,

On the sixth day the animals were killed and the zinc content of the islet cells were examined. It was found that, on each of the animals treated with any of the mentioned hormones, the zinc content of the islets distinctly augmented, most markedly with praehormon, next with thyradin, and relatively less with interenin.

Results of other series of experiment in which the animals were first treated with one of the mentioned hormones and then were injected with the diabetogenic reagents are given in table 3.

The results of these experiments have verified that, the administration of these hormones reduces strongly β cells of the islets of Langerhans by injection of the diabetogenic reagent, which accelerates the production of diabetes.

Here is again a correlation between the zinc content of the islets and production of experimental diabetes. In this case, it may also be counted to a factor that the islet cells fall into overwork by the action of these hormones, and then their resistance is reduced.

IV. Discussion

It was already reported by Griffiths (cited in 11, 12) that the difficulty of production of experimental diabetes on the guinea pig by means of injecting alloxan was considered to be due to more plentiful content of glutathion in the blood of this animal than other animals. Collins-Williams and others (11), however, reported that, when the guinea pig was fed with diet lacking in methionin and cystine, the glutathion content of its blood became lower than rabbits, yet, severe diabetes could not be produced experimentally.

On the other hand, it is possible to suppose that the resistance of guinea pig against experimental diabetes is due to its ability to regenerate vigorously and proliferate β cells. According to our experiments, however, when the islets of Langerhans of the guinea pig were examined in a few hours after administration of any of alloxan, oxine or dithizone, the damage of the islet cells is in a lighter degree than is observed on other animals. Hence, it is hard to comply with the opinion that the resistance of the guinea pig against experimentatal diabetes is ascribed to the ability of regeneration and proliferation of β cells, but it is believed from the present experimental results that this resistance of the guinea pig is due to the poor content

of zinc in the islet cells.

In short, the experiments reported in this paper furnish a sufficient support to the opinion already offered that the experimental diabetes caused by the diabetogenic reagent is due to a combination of the reagent to zinc contained in the islet cells of which the reagent thus combined acts as injurious.

Considering experimental results thus far advanced, it is certain that the content of zinc in the islets of Langerhans goes parallel with facility of producing experimental diabetes on animals. As is seen in Fig. 3, the zinc content of human islets of Langerhans is almost similar in degree to that of the rabbit's islets. From this fact it may by assumed that diabetogenic reagents, such as alloxan, oxine or dithizone when introduced to or produced in the human body, severe diabetes mellitus will result almost the same facility as is seen in the rabbit.

V. Summary

(1) It was demonstrated that in the production and the course of diabetes mellitus after the administration to animals of a diabetogenic reagent such as alloxan, oxine or dithizone, the degree in the production of diabetes mellitus, from severe to slight case, were in an order of rabbit, dog, rat, mouse, cat and guinea pig.

The fact has a parallel relationship with the quantity of zinc which is histochemically detected in the islets of Langerhans.

(2) As the degree in the production of dithizone diabetes of the infant rabbit becomes the more severe, the more it grows up. Finally about 40 days after the delivery (the weaning period) the degree in the production of diabetes mellitus reaches to that of adult rabbits. This fact has, too, shows a parallel relationship with the quantity of zinc in the islets of Langerhans.

(3) The increased quantity of zinc in the islets of Langerhans was observed after administration of anterior pituitary hormone, thyroid hormone, and adrenal cortical hormone, the production of diabetes mellitus being accelerated with the increased content of zinc.

Accordingly, it is obvious that the degree in the production of diabetes mellitus after the administration of diabetogenic reagents is intimately connected with the variations in quantity of zinc in the islets of Langerhans.

The zinc content of human islets of Langerhans is similar in the degree to that of the rabbit. From this fact the severe diabetes mellitus of human body may be caused as easily as in the rabbit by diabetogenic substances.

FEFERENCES

(1) Okamoto, K.: Transactions Societatis Pathologicae Japonicae, **32**, 99, 1942.

(2) Okamoto, K.: id., **33**, 247. 1943.

(3) Okamoto, K.: Acta Scholae Medicinalis Universitatis in Kioto, **27**, 43, 1949.
The Japanese Journal of Endocrinology. **25**, 32, 1949, (in Japanese).

(4) Kadota, I.: The Journal of Laboratory & Clinical Medicine, **35**, 568, 1950.

(5) Bailey, C. C.: Alloxan diabetes mellitus, 8. edition, 178, Lea & Febiger, Philadilphia, 1947.

(6) Duff, G. L.: The American Journal of the Medical Society, **210**, 381, 1945.

(7) Iizuka, N.: Consideration on diabetes mellitus. Appendix. Alloxan diabetes. Advance of clinic, **1**, 37, Nagai, Osaka, 1949, (in Japanese).

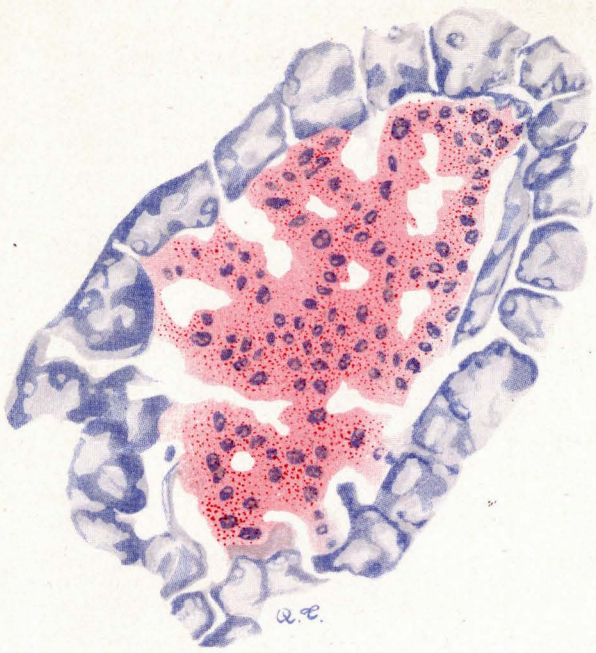
(8) Okinaka, S. a. J. Kosaka: Alloxan diabetes. Kinoshita's Advance of Medicine. **6**, 392, Nanjo, Tokyo, 1949, (in Japanese).

(9) Gomori, G.: The American Journal of Pathology, **15**, 497, 1939. id., **17**, 395, 1941.

(10) Okamoto, K. a. M. Hashimoto: The Japanese Journal of Constitutional Medicine, **13**, 83, 1944, (in Japanese).

(11) Collins-Williams, J., A. E. Renold a. A. Marble: Endocrinology, **46**, 1, 1950.

(12) Johnson, D. D.: id., **46**, 135, 1950.



← Fig. 1

Zinc in cells of the islet of Langerhans of the pancreas —normal rabbit.
(Stained by diphenylthiocarbazide method).

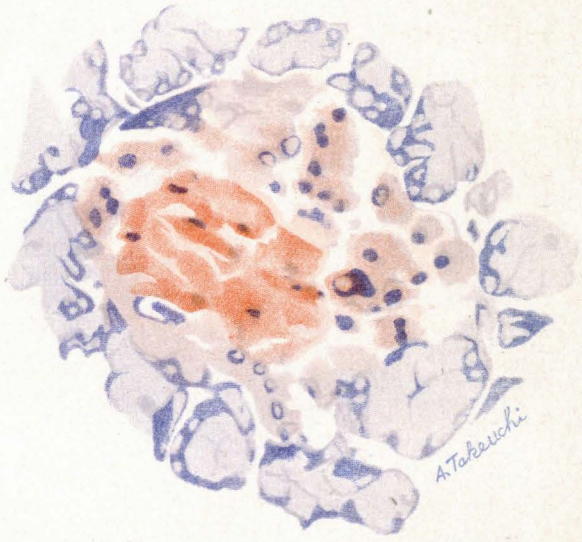
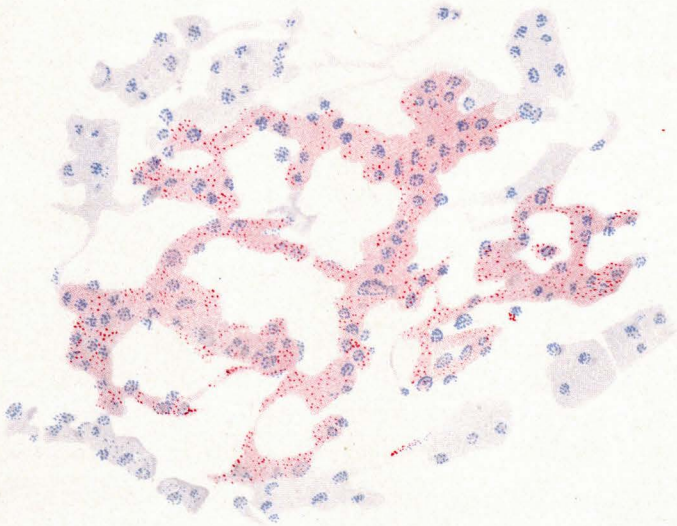


Fig. 2 →

Strong decrease of zinc in the cells and destruction of cells of the islet of Langerhans—rabbit, 16 hours after intravenous injection of oxine 50 mg/kg.
(Stained by diphenylthiocarbazide method).



← Fig. 3

Zinc in cells of the islet of Langerhans of pancreas –normal man.
(Stained by diphenylthiocarbazide method).