ZINC CONTENT IN ENDOCRINE AND EXOCRINE TISSUES OF THE PANCREAS

Ya. A. Lazaris, V. I. Lapin,
T. V. Pal'mina, and V. I. Korchin

UDC 612.34.015.31:546.47

A method of quantitative spectrographic estimation of zinc in the pancreas has been developed. The zinc content in the islets of Langerhans is considerably higher than its concentration in the exocrine tissue.

* * *

On the assumption that Zn is a structural component of insulin, its content in the pancreas has been investigated [3, 4]. The claim has been made that insulin, which is stored as granules in the β-cells of the islets, is bound with Zn, which also participates in its secretion [9]. In diabetes Zn metabolism, and possibly its content in the pancreas are modified [3, 4, 9, 11]. Zn^{65}, after entering the body, accumulates in the pancreas and is actively metabolized in the islets of Langerhans [4]. Qualitative histochemical tests have proved the presence of Zn in endocrine tissue in man, rabbits, dogs, horses, pigs, mice, rats, pigeons, and various fishes [2]. Investigation of the Zn content in the islets of certain teleost fishes, whose endocrine tissue is anatomically separate from the exocrine tissue, has shown that the Zn concentration in the endocrine tissue is much higher than in the exocrine [14]. In the islets of Langerhans of ducks and other birds, the Zn content is 10 times higher than in the remaining tissue of the gland [15].

Despite the fact that the great majority of investigations into the physiology and pathology of the incretory function of the pancreas have been carried out on mammals, there is no information in the literature concerning the content of Zn separately in their exocrine and endocrine tissues. This is because of the great difficulty encountered in obtaining islet tissues in quantities sufficient for estimation of their Zn content. Determination of the Zn in the whole gland, which contains only a small proportion (2-3%) of islet tissue, gives no indication of its concentration in the islets.

In several earlier investigations of the pathogenesis of diabetes [1, 2, 5-8] evidence was obtained of the important role of Zn in the incretory function of the mammalian pancreas. Furtherance of this study required determination of the quantitative content of Zn in the endocrine and exocrine tissue. The investigation described below marks the first steps in this direction.

EXPERIMENTAL METHOD

Dithizone, made up in 0.25% ammonia solution, was injected intravenously in a dose of 100 mg/kg body weight into noninbred rabbits after fasting for 24-48 h. The animals were killed by air embolism 5-10 min after the injection. The pancreas was quickly removed and fixed by the usual method in alcohol saturated with hydrogen sulfide [12, 13]. After the usual treatment, the dewaxed sections were stained to detect Zn with dithizone and 8-(toluenesulfanilamino)-quinoline by the writers' own method [2]. To detect Zn in the exocrine tissue at the same time, the method of spodography [12] was used, i.e., histochemical determination of Zn after incineration of the sections. Sections of the pancreas embedded in paraffin wax, 10 μ in thickness, were placed on slides and incinerated in a muffle furnace at a temperature not exceeding 450°. The sections retained their structure, and they were stained by the same reagents as in the previous method.

A method developed by the writers was used for the quantitative estimation of Zn. A solution of dithizone was injected intravenously into the rabbits and the animals were sacrificed 5-10 min later. The pancreas was removed and weighed, and immersed in liquid oxygen. It was then thoroughly minced, and

Department of Pathological Physiology, Karaganda Medical Institute. (Presented by Academician V. V. Parin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 2, pp. 52-55, February, 1970. Original article submitted August 18, 1969.

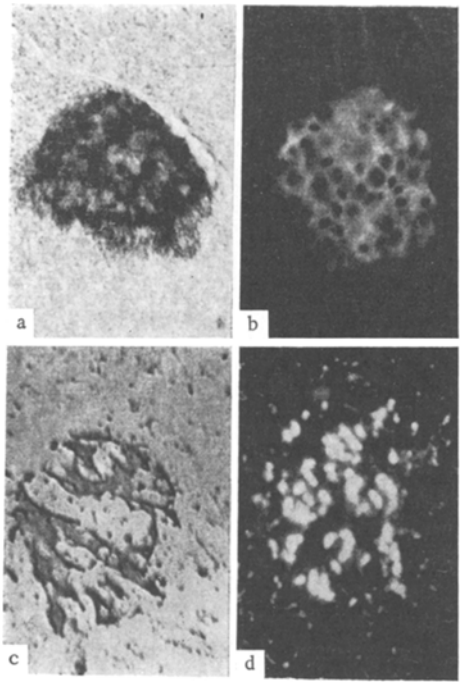

Fig. 1. Positive histochemical reaction for zinc in islets of Langerhans of the pancreas. Reaction with dithizone (a) and with 8-(toluenesulfanilamino)-quinoline (b) in section of pancreas; reaction with dithizone (c) and with 8-(toluenesulfanilamino)-quinoline (d) in incinerated section through pancreas (spodogram). 180×.

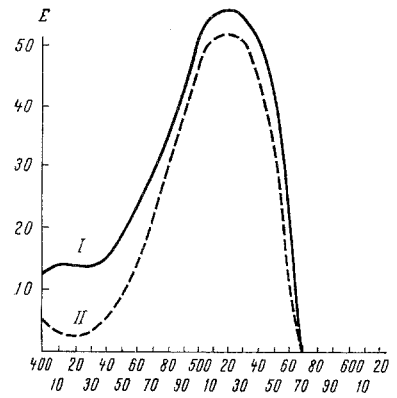

Fig. 2. Absorption spectrum of chloroform extract of pancreas after intravenous injection of dithizone (I), and absorption spectrum of solution of chemically pure zinc dithizonate (II).

the colored Zn dithizonate formed in the islets was extracted from the homogenate by means of chloroform until a colorless extract was obtained. The whole pooled extract was evaporated and its Zn content was determined spectrographically by means of the DFS-13 diffraction spectrograph. As a preliminary step, the absorption spectrum of the chloroform extract was investigated on the SF-4 spectrophotometer and compared with chemically pure Zn dithizonate. Zn in the whole gland was determined spectrographically after complete incineration.

EXPERIMENTAL RESULTS

Histochemical tests on the pancreatic sections revealed Zn clearly in the islets of Langerhans (Fig. 1a, b). The test proposed by the writers [2] can be regarded as a specific reaction. The reaction for Zn was negative in exocrine tissue. Evidently Zn is present in the exocrine and endocrine tissues in the form of various compounds with proteins. Some of them (in islets) react with reagents for Zn, while others (in exocrine tissue) do not behave in this way. That is why, to detect the presence of Zn in exocrine tissue, the incineration method had to be used [12]. As a result of this procedure, after breakdown of the organic matter, the metal was liberated. Treatment of the sections in this way showed that both endocrine and exocrine tissues give a positive reaction for Zn. However, most metal was found in the islets, in which its concentration was higher than in the exocrine tissue (Fig. 1c, d). Zn in the secretory tissue is a component of carboxypeptidase and, possibly, other enzymes.

The results of these experiments (Fig. 1), while they indicate a much higher concentration of Zn in the islets, do not provide a complete picture of its content in the endocrine and exocrine tissues. In order to solve this problem, the content of the metal had to be determined in each tissue separately. It is practically impossible to isolate sufficient endocrine tissue by excision of the islets of Langerhans. Another way was therefore selected for the quantitative determination of Zn in the incretory tissue. The suggested method consisted essentially of removal of the Zn dithizonate formed in the islets after administration of dithizone into the animals, and which is absent in exocrine tissue (Fig. 1a), by means of chloroform from the endocrine tissue. Zn in the extract thus obtained was determined spectrographically. Before carrying out the quantitative estimation of Zn, it was advisable to make sure that the extract obtained from the homogenized tissue in fact contained Zn dithizonate. To make certain of this, the absorption spectrum of a chloroform extract of the pancreas, which was redish violet in color, was investigated. It was compared with the absorption spectrum of a chloroform solution of chemically pure Zn dithizonate. It is clear from Fig. 2 that the absorption spectra of both solutions showed a very close resemblance both in the character of the curves and in the coincidence of their maxima. The results of this experiment, in agreement with findings obtained by other workers [11, 16], indicate the presence of Zn dithizonate in extract from homogenate of the pancreas.

TABLE 1. Zn Content in Islet Tissue of Rabbit Pancreas

Number of animals in experiment	Mean weight of pancreas (in mg)	Mean content of islet tissue in pancreas (in mg)	Zn content (in μg)	
			in islet tissue of one pancreas	in 1 g islet tissue
5	484	14.52	4.48	308.5
11	504	15.12	2.58	170.2
5	564	16.92	7.30	431.4
5	484	14.52	7.50	516.5
M±m	509±19.4	15.27±0.58	5.46±1.19	356.0±84.0

For the quantitative determination of Zn in the pancreatic endocrine tissue, four groups of experiments were carried out on 26 rabbits. Five animals were used in each of three groups, and 11 animals in one group. The mean values of the Zn content obtained in each group are given in Table 1. They were calculated per gram of fresh islet tissue, assuming a 3% content of zinc in the whole pancreas.

It is clear from Table 1 that the Zn content in the islets of Langerhans varied, from 170.2 to 516.5 μg/g, with a mean value of 356.0 μg/g. Zn was also determined quantitatively in the whole pancreas of 15 rabbits. Its mean content (M±m) was 48.0±2.76 μg/g fresh pancreatic tissue. Comparison of the Zn content in the incretory tissue and in the whole gland shows conclusively that its concentration in the islet tissue is 7.4 times higher than its concentration in the whole gland.

The discovery of a high concentration of zinc in the pancreatic islets of Langerhans in rabbits is strong confirmation of the hypothesis regarding the important role of Zn blocking in the pathogenesis of the numerous experimental models of diabetes produced by the writers in this species of animal [1, 2, 5-8].

The results of these experiments, in conjunction with those obtained by other workers [4], provide a solid basis for the further study of Zn metabolism in human diabetes.

LITERATURE CITED

1. Z. E. Bavel'skii, V. M. Dziomko, I. A. Krasavin, et al., Pat. Fiziol., No. 3, 51 (1968).
2. I. A. Krasavin, Z. E. Bavel'skii, Ya. A. Lazaris, et al., Probl. Éndokrinol., No. 3, 102 (1969).
3. Ya. A. Lazaris, Pat. Fiziol., No. 4, 83 (1959).
4. Ya. A. Lazaris, Pat. Fiziol., No. 5, 75 (1960).
5. Ya. A. Lazaris, Probl. Éndokrinol., No. 2, 77 (1966).
6. Ya. A. Lazaris and A. Ya. Lazaris, Probl. Éndokrinol., No. 2, 75 (1967).
7. Ya. A. Lazaris and A. Ya. Lazaris, Byull. Éksperim. Biol. i Med., No. 7, 45 (1967).
8. Ya. A. Lazaris, V. M. Dziomko, and I. A. Krasavin, Probl. Éndokrinol., No. 4, 107 (1968).
9. H. Maske, Diabetes, 6, 335 (1957).
10. B. Stampfl, Acta Histochem. (Jena), 8, 406 (1959).
11. S. Tarui, Endocr. Jap., 10, 9 (1963).
12. F. Timm, Dtsch. Z. Ges.,Gerichtl. Med., 47, 428 (1958).
13. Y. Voigt, Arch. Path. Anat., 295, 332 (1959).
14. G. Weitzel, F. J. Strecker, and U. Roester, Arch. Path. Anat., 295, 83 (1953).
15. G. Weitzel, E. Buddecke, and D. Kraft, Hoppe-Seylers Z. Physiol. Chem., 305, 9 (1956).
16. H. Wolff and D. Ringleb, Z. Ges. Exp. Med., 124, 236 (1954).