ZINC CONTENT IN ISLET CELLS OF THE MAMMALIAN PANCREAS IN RELATION TO THE FUNCTIONAL STATE OF THE INSULAR SYSTEM

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The zinc concentration in the A and B cells of the islets of Langerhans was determined quantitatively in various mammals (rabbits, golden hamsters, mice, rats, cats, dogs) and also in rabbits with various states of the insular system caused by starvation, injections of glucose, and administration of the diabetogenic agent dithisone. A relationship was established between the blood glucose concentration and the zinc concentration in the B cells. The results of these experiments confirm the conclusion that zinc plays a role in the mechanism of insulin secretion.

Investigations of zinc in the A and B cells of the islets of Langerhans in different functional states have been carried out previously only with the aid of qualitative histochemical methods [2, 6-10]. Where attempts have been made to determine zinc quantitatively this has been only in the islet tissue as a whole and not separately in the different types of cells, which are known to subserve different functions in the body [3, 4, 11].

The object of this investigation was to develop a method of determining the zinc content separately in the A and B cells of the islets of Langerhans and to study the zinc content in these cells in animals of different species and in different functional states of the endocrine apparatus of the pancreas.

EXPERIMENTAL METHOD

The experimental animals were 45 rabbits, eight golden hamsters, 13 mice, 10 cats, eight dogs, and 11 rats. Eight rabbits were starved for 48 h, seven rabbits received a single injection and five others received six injections of glucose, each in a dose of 10 g/kg as a 40% solution, and diabetes was induced in another 12 rabbits by intravenous injection of dithisone in a dose of 50 mg/kg made up in 0.25% ammonia solution.

TABLE 1. Zinc Content in A and B Cells of Islets of Langerhans in Various Mammals (M ± m)

Animals	No. of animals	Zinc content in pancreas (μg/g dry weight)	
		cells A	cells B
Rabbits	13	5,2±0,5	53,3±3,6
Hamsters	8	0,7±0,4	38,5±2,1
Mice	13	4,4±0,5	28,8±1,9
Cats	10	2,6±0,3	17,2±0,9
Dogs	8	1,7±0,2	11,7±0,7
Rats	11	5,9±1,0	17,9±1,8

The blood sugar was estimated by the Hagedorn–Jensen method. The animals were killed at the end of the period of starvation, 2 h after the last injection of glucose, and 5-15 days after administration of dithisone.

The pancreas was divided into three parts. One part was homogenized, and the zinc present in the islets was ex-

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TABLE 2. Zinc Content in A and B Cells of Islets of Langerhans in Intact Rabbits, Starved Rabbits, Rabbits Receiving Glucose, and Rabbits with Dithisone Diabetes (M ± m)

Group of animals	No. of animals	Blood sugar (mg %)	Zinc content in pancreas (μg/g dry weight)	
			cells A	cells B
Intact	13	102,2±2,1	5,2±0,5	53,3±3,6
Starved	8	74,4±6,7	4,1±0,5	75,9±4,4
P		<0,001	>0,1	<0,001
Receiving glucose:				
10 g/kg	7	505,1±32,4	6,8±1,2	34,5±4,0
P		<0,001	<0,001	<0,001
10 g/kg × 6	5	527,4±36,6	7,8±1,6	15,2±2,1
P		<0,001	<0,001	<0,001
With diabetes	12	275,0±13,5	8,7±0,5	2,5±0,3
P		<0,001	<0,001	<0,001

tracted from the homogenate by means of a 0.1% solution of dithisone in acetone. Its concentration was determined by the highly sensitive luminescence method [5].

Another part of the pancreas was fixed by Timm's method and paraffin sections cut with a rotating microtome were used to obtain the luminescence histochemical reaction for zinc by the method described elsewhere [7]. Evidence of the quantitative nature of this reaction is given by the absence of metachromasia and of luminescence of the preparations under the influence of ultraviolet light during microscopic investigation, the low optical density of the object (less than 0.2 optical density unit), and the fact that the dye fixed to the tissue obeys the Bouguer–Lambert and Beer laws. Microfluorimetry was carried out by the method suggested by Varskii and Khavkin [1]. To identify the A and B cells in the islets of Langerhans, sections with a luminescence reaction were counterstained with hematoxylin-phloxin and with aldehyde-fuchsin [2].

The third part of the pancreas was fixed in Bouin's fluid and serial paraffin sections were stained with aldehyde-fuchsin and with hematoxylin-phloxin by Gomori's method. The area occupied by the A and B cells in the islets of Langerhans was measured on the sections in relative units. For this purpose, a transparent disc on which a grid was marked was placed in the focal plane of the ocular.

The zinc content was calculated separately in the A and B cells of the islets of Langerhans in μg/g dry weight of the gland by the authors' formula from the results of chemical analysis, microfluorimetry, and measurement of the areas.

EXPERIMENTAL RESULTS

The results of determination of the zinc concentration in the islet cells of intact mamals are given in Table 1. The zinc concentration in the B cells was greatest in rabbits, smaller in hamsters and mice, and smaller still in rats, cats, and dogs. Compared with cats and dogs, these cells in rats give paler luminescence, but since they are much more numerous in the islets than in the first two species of animals, their total zinc content is greater.

The zinc content in the A cells diminished in the mammals in the following order: rats, rabbits, mice, cats, dogs, hamsters (Table 1). Despite the intensive luminescence of these cells observed in rabbits and rats, the total zinc content in these cells was much less than in the B cells because of the small volume which they occupy in the islets of Langerhans.

In all the mammals investigated the greater part of the zinc was thus concentrated in the B cells. Its unequal content in animals of different species is evidently connected with differences in the state of their carbohydrate metabolism.

It will be clear from Table 2 that starvation is accompanied by considerable accumulation of zinc in the B cells. Its content in these cells was greatly reduced after administration of glucose, especially if repeated. The zinc content in the B cells of the islets of Langerhans was sharply reduced in the rabbits with diabetes. Fluctuations in the zinc content in the A cells under the various experimental conditions were less marked. After starvation the zinc content in the A cells differed only slightly from normal, while after glucose loading and in diabetes it was increased.

Comparison of the blood sugar with the zinc content in the cells of the islets revealed a definite inverse relationship between the blood glucose and the zinc content in the B cells of the islets, but no such definite relationship could be found for the A cells.

It can be concluded from these results that zinc plays an important role in the endocrine function of the pancreas and, in particular, in the mechanism of insulin secretion. Zinc atoms evidently control the blood sugar level through the secretion of the hormone. The zinc content in the islet cells can be taken as an index of their functional state.

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