EFFECT OF DECOCTION OF LINSEED (Linum usitatissimum L.) ON MORPHOLOGICAL AND FUNCTIONAL CHANGES IN PANCREATIC CELLS IN LATENT ALLOXAN DIABETES

G. N. Voronin and Z. P. Kasumova

UDC 615.322:582.751.42-148].014.24.017.272.3:612.34.014.2 + 616.379-008.64-092.9-085.322:582.751.42-148]-07:616.37-018

Decoction of linseed, used as a biological stimulant, improved the general conditions of rats with a latent form of alloxan diabetes. Under the influence of this decoction, proliferation of exocrine cells in the pancreas was stimulated resulting in the development of new β cells. Secretion formation was increased in the β cells.

* * *

Reports of the hypoglycemic action of decoctions and extracts of plants (linseed, bilberry, haricot bean, nettle, etc.), with their successful use in the clinical treatment of mild forms of diabetes mellitus, have recently appeared with increasing frequency in the literature [1, 6-11]. No morphological investigations of the effect of these plant substances on the pancreatic tissues in diabetes could be found in the accessible literature.

Our previous investigations [2, 3, 5] showed that during development of alloxan diabetes in the pancreas in rats, death of β cells is accompanied by their partial replacement by new β cells formed by mitotic division and by development from central acinar cells and also from cells lining the small efferent ducts.

In the present investigation we attempted to stimulate proliferation in the pancreas in rats with a latent form of alloxan diabetes, using decoction of linseed (Linum usitatissimum L.) as biological stimulant.

EXPERIMENTAL METHOD

Four series of experiments were carried out on 24 male albino rats weighing 269-272 g, with 6 animals in each series. Alloxan was injected subcutaneously into the rats in a dose of 5 mg/100 g body weight daily for 4.5 months. After a double glucose tolerance test (by the method of Exton and Rose) had revealed functional insufficiency of the β cells, some of the animals began to receive a freshly prepared 10% decoction of linseed by mouth in a dose of 0.1 ml once daily for 2.5 months for therapeutic purposes. All the rats were kept on the same diet. Throughout the experiment (4.5 months) observations were kept on the general condition of the animals, their weight, their level of food excitability, and their blood sugar. Pieces of pancreas from the splenic portion were fixed in Bouin's fluid. Paraffin sections were stained with hematoxylin-eosin, with azocarmine by Gomori's method, and with paraldehyde-fuchsin by the Gomori-Gabe method. In histological preparations of the gland (area of section 6.4 mm^2) the number of islets was counted and their area measured. The number of mitoses was counted in cells of the islets, acini, intermediate areas, and efferent ducts.

EXPERIMENTAL RESULTS

Administration of linseed decoction to healthy rats (Table 1, series II) had no significant effect on their weight or blood sugar. The biostimulant considerably increased the mitotic activity of their acinar

K. M. Bykov Department of General Physiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR S. V. Anichkov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 10, pp. 112-115, October, 1968. Original article submitted July 13, 1967.

TABLE 1. Changes in Islets of Langerhans under the Influence of Decoction of Linseed (Linum usitatissimum L.) Expressed per Area of Pancreas of 6.4 mm² (mean data)

Series of expts.	Animals	No. of islets of Langerhans				No. of islet cells		Endocrine tissue (in μ^2)	Functional index
		small, from 3-5 to 1700 μ^2	medium sized, from 1700 to 15,000 μ^2	large, from 15,000 to 73,200 μ^2	total	β cells	α cells		
I	Healthy rats receiving 0.1 ml physiological saline once daily	6±1.1	5±1.2	2±0.5	13±1.1	737±28.3	142±39.6	0.142±0.018	5.1
II	Healthy rats receiving 0.1 ml decoction of linseed once daily	6±1.5	7±1.7	2±0.4	15±2.8	618±17.8	126±34.5	0.137±0.018	4.9
III	Rats receiving siloxan for 4.5 months	7±1.1	5±0.3	1±0.3	13±1.8	207±25.8	74±14.7	0.045±0.008	2.8
IV	Rats receiving siloxan and linseed decoction once daily	8±1.4	6±0.6	2±0.3	16±1.7	360±35.8	138±22.4	0.082±0.004	2.6

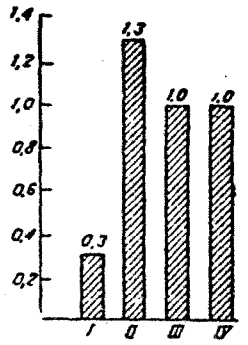

Fig. 1. Mitotic activity of pancreatic acinar cells of rats. Ordinate, mitotic index (in %). Roman numbers denote serial numbers of experiments.

cells compared with that of healthy animals not receiving decoction of linseed (Fig. 1, series II). No other changes could be found in the pancreatic cells.

In animals receiving alloxan injections (Table 1, series III) the mean blood sugar level fluctuated within normal limits (85-107 mg%). Despite this, the rats showed morphological changes in the pancreas. Small islets of Langerhans predominated in the gland. In some medium-sized and large islets the cell borders of the β cells could not be clearly distinguished and their trabecular character of arrangement was disturbed. In the injured β cells, various stages of degranulation of the cytoplasm were observed (Fig. 2a) As Fig. 2 shows, some β cells (β') did not contain secretion, while others (β'') contained small amounts uniformly distributed throughout the cytoplasm, and a third type of β cells (β''') contained significant amounts of secretion only in certain parts of the cytoplasm. The α cells in the islets were not destroyed. However, the ratio between β and α cells was changed: the functional index was reduced to approximately two-thirds of that for healthy animals (Table 1).

The excretory function of the pancreas in the rats in the experiments of series III was evidently undisturbed, because zymogen granules were found in most acinar cells and secretion in the efferent ducts. In some parts of the gland a moderate hyperemia and edema of the interlobular connective tissue were observed.

In the animals of this series the mitotic activity of the acinar cells was approximately three times higher than that of healthy rats receiving physiological saline (Fig. 1, series III). Meanwhile proliferation of the central acinar cells and cells of the efferent duct system was increased. We could not determine the intensity of mitotic activity of the β cells by counting mitoses either in the healthy or in the sick animals, because of their small numbers.

In most rats receiving linseed decoction (series IV) the blood sugar was about the same as in the untreated diabetic animals. An increase in the amount of islet tissue was observed in the rats after administration of the biostimulant (Table 1, series IV). The ratio between β and α cells in the islets remained almost unchanged. Secretion formation in the β cells was considerably increased after treatment of the animals (Fig. 2b).

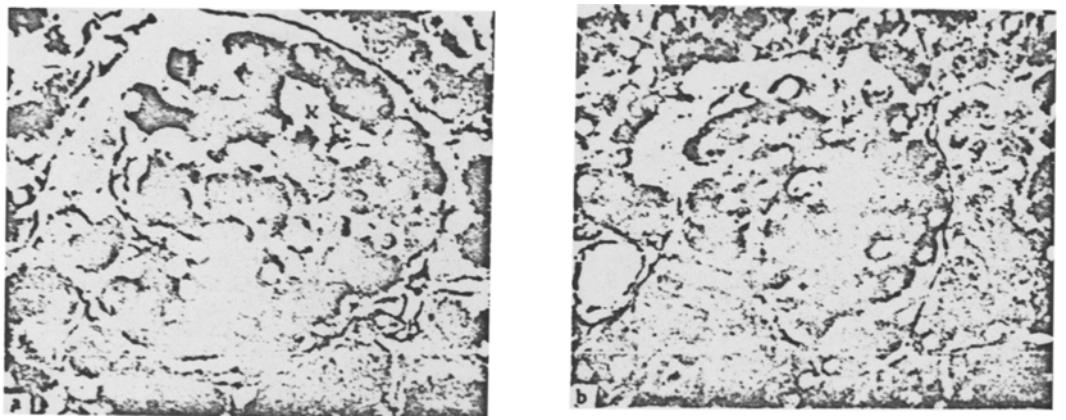

Fig. 2. Pancreas of untreated rat (a) and of rat after treatment with linseed decoction (b). a) Modified β cells with varied amounts of secretion in an islet of Langerhans (area 3980 μ^2). Acini with small number of zymogen granules around the islet; b) nearly all β cells in an islet of Langerhans (area 8750 μ^2) contain large amounts of secretion, more or less uniformly distributed throughout the cytoplasm. α cells, not stained dark with paraldehyde-fuchsin on the photograph, can be seen at the top of the islet as a pale band. Below on the left, an empty efferent duct. Fixation with Bouin's fluid, stained with paraldehyde-fuchsin. 600×.

Neither hyperemia nor edema were observed in the pancreas of this group of animals. The acinar cells were in an active state. In the cells of the intermediate zones and efferent ducts reactive processes were constantly found, shown by marked swelling of the nuclei and mitotic division. The number of mitoses in the acinar cells was the same as in the rats of the preceding series and was much higher than the mitotic index in healthy animals receiving physiological saline only (Fig. 1, series IV).

Decoction of linseed thus improved the general state of rats with a latent form of alloxan diabetes, stimulating proliferation not only of undifferentiated cells, but also of acinar cells in the pancreas, thereby leading to the developing of new islets of Langerhans. In addition, this biostimulant promoted secretion formation in the β cells. The hypoglycemic action of linseed decoction was not exhibited in rats with the latent form of alloxan diabetes. However, in rats with a manifest form of this disease, linseed decoction lowered the blood sugar [4].

LITERATURE CITED

1. G. N. Bezdetko, in: Collected Proceedings of the Third Scientific Conference of Physiologists, Biochemists, and Pharmacologists of the Western Siberian Group of the I. P. Pavlov All-Union Physiological Society [in Russian], Tomsk (1965), p. 281.
2. G. N. Voronin, Z. P. Mikhal'skaya, and A. V. Rikkl', Byull. Éksperim. Biol. i Med., No. 12, 103 (1962).
3. G. N. Voronin and Z. P. Kasumova-Mikhal'skaya, Researches in Tissue Biology [in Russian], Vol. 1, Tartu (1966), p. 54.
4. Z. P. Kasumova, in: Mechanisms of Nervous and Humoral Regulation of Physiological Processes [in Russian], Leningrad (1966), p. 29.
5. Z. P. Kasumova, in: Proceedings of a Conference of Undergraduate and Postgraduate Students of Departments of Morphology and Laboratories of Leningrad Higher Educational and Research Institutes [in Russian], Leningrad (1966), p. 18.
6. P. T. Kondratenko, S. D. Kur, and P. M. Rozhko, Preparation, Growth, and Treatment of Medicinal Plants [in Russian], Moscow (1965).
7. V. P. Makhlayuk, Medicinal Plants in Popular Medicine [in Russian], Saratov (1964).
8. N. K. Smol'yaninova, in: Plants with Domestic Uses [in Russian], Moscow (1963), p. 7.
9. P. D. Sokolov, in: Useful and Harmful Plants of Leningrad Region [in Russian], Leningrad (1963), p. 53.
10. V. V. Solov'ev and L. F. Perova, in: Proceedings of the Second Conference on Investigation of Medicinal Plants of Siberia and the Far East [in Russian], Tomsk (1961), p. 86.
11. V. N. Shukhardin, Forest Drugs. Wild Medicinal Plants [in Russian], Perm' (1966).