may be explained by a predominance of factors stimulating cell proliferation and thus making the cells insensitive to the inhibitors.

From the results of the experiments it can be concluded that the sensitivity of kidney cells remain practically unchanged from pubescence to old age, in contrast to the liver cells and vaginal epithelium, which exhibit a distinct age dependence [5, 11]. The constancy of the kidney cells' sensitivity in mature and old animals allows the assumption to be made that at this regulatory level the organism controls the proliferation with a sufficient stability.

REFERENCES

1. V. N. Anisimov, in: *Chalones: Significance and Role in Normal and Pathological Processes* [in Russian], Moscow (1981), pp. 43-46
2. V. N. Anisimov, in: *International Symposium on Chalones,* Abstracts, Moscow (1983), pp. 114-116.
3. A. Balazh and I. Blazhek, *Endogenous Inhibitors of Cell Proliferation* [in Russian], Moscow (1982).
4. S. A. Ketlinskii and E. V. Parfenova, *Byull. Eksp. Biol.*, **92**, № 7, 96-98 (1981).
5. A. I. Klimenko, M. Ya. Shevtsova, A. B. Malyshev, and V. V. Gautsel', *Biokhimiya,* **53**, 979-984 (1988).
6. V. B. Okulov and L. I. Chekulaeva, *Arkh. Anat.*, № 1, 106-109 (1976).
7. Yu. A. Romanov, S. A. Ketlinskii, A. I. Antokhin, and V. B. Okulov, *Chalones and Regulation of Cell Division* [in Russian], Moscow (1984).
8. M. G. Trudolyubova, in: *Current Methods in Biochemistry* [in Russian], Moscow (1977), pp. 313-316.
9. W. S. Bullough, and E. B. Laurence, *Proc. Roy. Soc. B.*, **151**, 517-536 (1960).
10. O. H. Lowry, N. J. Rosebrough, A. J. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
11. H. A. Saetren, *Exp. Cell Res.*, **11**, № 2, 229-232 (1956).

MORPHOLOGY AND PATHOMORFOLOGY

Blood Serum Factors from Rabbits with Acute Pancreatitis as Stimulators of Regeneration of B-Cells of the Pancreatic Islets in Experimental Diabetes

V. P. Zharkov, V. N. Yarygin, and A. A. Dolzhikov

UDC 616.379−008.64−092.9−085.366.15−092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 5, pp. 525−527, May, 1993
Original article submitted December 22, 1992

Key words: *experimental diabetes; B-cells; regeneration; humoral factors*

One of the most promising ways of regulating the regeneration processes is to utilize natural endogenous humoral modulators or to stimulate their synthesis in the organism. This aspect of the problem has been studied in most detail with regard to liver regeneration [1,2,5]. At the same time, the role of humoral factors in the initiation of reparative regeneration of the pancreatic endocrine tissue is poorly understood.

Department of Histology, Kursk Medical Institute; Department of Biology, Russian Medical University, Moscow

0007-4888/93/0005-0577$12.50

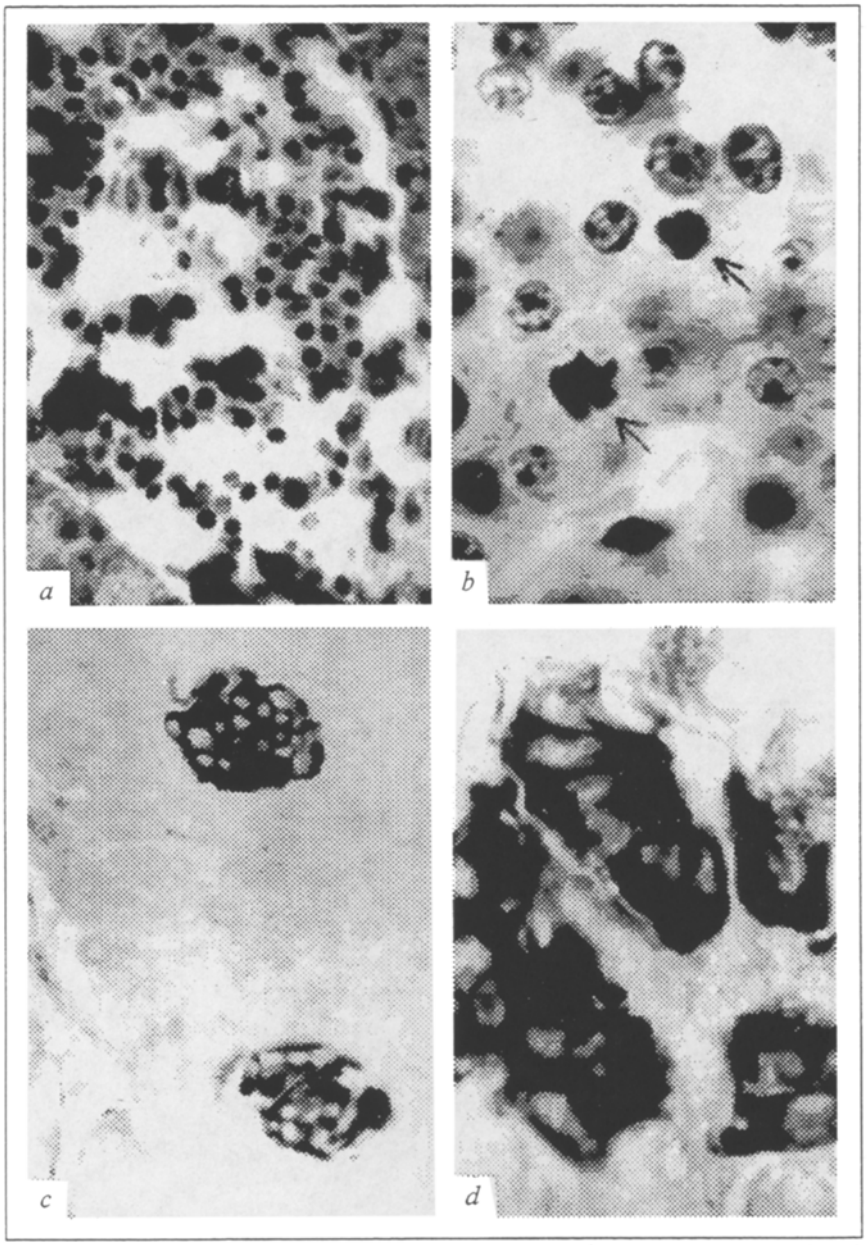

Fig. 1. Morphological changes in the pancreatic islets in rabbits with alloxan–induced diabetes after injections of serum. *a*) 7 days after alloxan injection without serum treatment (necrosis of B–cells); *b*) 24 hours after 3 injections of 12–hour serum (metaphases of endocrine cells); *c*) 7 days after injection of 12–hour serum (newly formed associates of B–cells), *d*) 21 days after injection of 12–hour serum (B–cell formation in the acini). Staining with hematoxylin and eosin (*a, b*) and aldehyde–fuchsin after Gomori (*c, d*); magnification: *a, c*) 280; *b, d*) 630.

The aim of the present study was to investigate the role of serum factors from animals with acute pancreatitis in the regulation of B-cell regeneration in alloxan-induced diabetes mellitus.

MATERIALS AND METHODS

Forty seven male and female Chinchilla rabbits weighing 2.0-2.5 kg were used for the experiment. Experimental diabetes was induced by single subcutaneous injection of alloxan in a dose of 200 mg/kg. After 7 days the animals were divided into 3 groups. The animals of the first group received i.v. injections of 5 ml/kg serum obtained from rabbits with acute pancreatitis 4, 12, and 24 hours after mechanical trauma of the pancreas; the second and third groups were injected with serum from sham-operated and intact rabbits, respectively. The serum was injected once a day during 3 days. The animals were killed by air embolization 1, 3, 7, 14, or 21 days after the last injection. Histological slices of the pancreas were stained with plain (hematoxylin and eosin, iron hematoxylin) and histochemical (aldehyde fuchsin, Phenaph dye [3], methyl-green - pyronin after Brasheugh) dyes. The mitotic index (MI) (in ‰) was estimated and the fasting level of glucose was determined in the blood by the orthotoluidine method and in the urine by the semiquantitative Glucotest method.

RESULTS

The biochemical and morphological changes observed 7 days after alloxan injection point to the development of acute diabetes mellitus. Hyperglycemia during fasting reaches 27.5 mmol/liter (the normal value is 4.3 mmol/liter), glucosuria being 6.5%. In the pancreatic islets dystrophy and necrosis of the B-cells are observed. The cytoplasm is condensed, the specific granulation disappears, and the nuclei undergo pycnosis and rhexis. The chains of B-cells break up, and their connections with hemocapillaries are broken. A large number of B-cells cord undergo lysis, accompanied by the formation of an amorphous unstructured mass (Fig. 1, *a*).

When rabbits are injected with serum from intact or sham-operated animals, no stimulation of the regeneration processes is observed at any of the time points. The intensity of proliferation varies from 0.01 to 0.04‰, which is typical for incretory cells of the intact adult organism. Histological changes in the islet apparatus are identical to those observed in diabetes mellitus in untreated animals.

Serum from the animals with mechanical trauma of the pancreas exhibits a stimulatory effect on B-cell

regeneration. This effect was found to depend on a number of factors: 1) the number of injections; 2) the time elapsing after the trauma; 3) the time after serum injection. The question whether the serum exhibits a species specificity merits special attention.

Only the first 2-3 injections of serum obtained one day after the pancreatic trauma possess a stimulatory effect. Increasing the number of injections up to 5 is not accompanied by enhancement of the mitotic activity.

The most pronounced stimulatory effect was observed one day after the last injection, the maximum being achieved with 12-hour serum (Fig. 1, *b*). Twenty-four hours after serum injection the mitotic activity of B-cells was 2.10‰ with 4-hour serum and 2.15‰ with 12-hour serum. The lowest effect was observed with 24-hour serum (MI as low as 1.13‰). The above differences became more pronounced during the subsequent period characterized by a weakening of the serum effect. For the injection of 4- and 24-hour serum, MI dropped to the control value as soon as on the 3rd day, whereas with 12-hour serum this index remained elevated as long as 7 days after the injection (1.09‰ and 0.76‰ on the 3rd and 7th day, respectively). As well as by the morphological data, the beneficial effect of the serum used is indicated by the glycemia level. In control animals with diabetes the blood glucose level steadily rose during the whole period of the experiment. The injection of serum from animals with pancreas trauma caused its stabilization and reliable reduction from the 9th to the 14th day to a constant value (16.5±1.8 mmol/liter).

At later stages of the experiment the B-cell mitotic activity decreases to control values. However, the compensatory processes in the insular tissue still continue. Among the islets small ones are predominant. Many of the islets consist solely of B-cells (Fig. 1, *c*); they are variously shaped and are often situated in acini of the exocrine part of the pancreas. The constant presence of cells with specific aldehyde-fuchsinophilic granulation in the acini (Fig. 1, *d*) and intercalary ducts suggests a pronounced acino-insular transformation in the pancreas of the experimental animals.

Thus, the rabbit serum collected after mechanical trauma of the pancreas possesses a short-term stimulatory effect on the proliferation of B-insulocytes surviving under conditions of alloxan-induced diabetes mellitus. This effect is maximally pronounced during the first 24 hours after 3 injections of 12-hour serum. At later times, the compensatory processes, appearing as acino-insular transformation, become predominant, while the mitotic activity is reduced. The nature of the stimulatory factors may be related either to the pancreatic hormones insulin and glucagon [4,6], or to immunomodulatory factors appearing in the blood upon the loss of or injury to an organ, as was shown under conditions of hepatectomy or toxic injury to the liver [1, 2].

REFERENCES

1. A. G. Babaeva, *Immunological Mechanisms in the Regulation of Regenerative Processes* [in Russian], Moscow (1976).
2. V. P. Zharkov, V. S. Barsukov, and V. D. Lun'kov, *Arkh. Patol.*, № 8, 69 (1990).
3. A. I. Konoplya, *Dep. in VINITI*, № 6430-85, Kursk (1985).
4. J. A. Caruana and A. A. Gage, *Surg. Gynec. Obstet.*, **150**, 390-394 (1980).
5. B. Fischer, P. Szuch, and M. Levine, *Science*, **171**, 575-577 (1971).
6. J. B. Price, *Metabolism*, **25**, № 11, Suppl. 1, 1427-1428 (1976).