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PROLIFERATION OF THE EXOCRINE AND ENDOCRINE PORTIONS OF THE PANCREAS AFTER ITS RESECTION

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After resection of the pancreas (about 40% of the weight of the organ) in (CBA × C57BL/6) hybrid mice weighing 20-28 g of the regenerative ability of the organ was found to be weak. After 21 days of the experiment no appreciable recovery of the weight of the organ had taken place. An increase in proliferative activity (the number of mitoses and the diurnal fraction of cells labeled with [^{3}H]thymidine were counted) was transient in character and did not extend to all the organ. The greatest number of labeled nuclei in the epithelium of the acini and islets was found in the region near to the site of injury, where the tissue of the organ was a little edematous. In areas not far from the wound surface but remaining unchanged the number of labeled cells was increased only in the early period after the operation. In regions of the organ remote from the site of injury (the duodenal loop) the number of labeled cells in the islets and acini was the same as in the control. The number of labeled cells in the islets was greater than in the acini.

KEY WORDS: mouse pancreas; islets and acini; proliferation; autoradiography.

Proliferative processes in the pancreas after resection of the organ have been inadequately studied. Most investigations have been conducted on rats and their object has been to study proliferation only of the exocrine part of the organ [1, 4, 7, 10, 12, 14]. Only in isolated investigations, also conducted on rats, has the important role of proliferative processes in the pancreatic islets been demonstrated [8, 13]. A few other investigations in this field made use of [^{3}H]thymidine [5, 6], but unfortunately they did not take into account the diurnal rhythm of cell division. The dependence of proliferative activity of the tissue of the gland remaining after resection on its remoteness from the site of injury has virtually not been studied.

The object of this investigation was to make a more detailed study of the proliferative activity of the epithelium of islets and acini of the pancreas after resection of the organ. The daily fraction of cells taking part in proliferation was determined. The index of labeled and dividing cells was calculated in three zones of the pancreas located at different distances from the site of trauma.

EXPERIMENTAL METHOD

Experiments were carried out on hybrid (CBA × C57BL/6) male mice with a mean weight of 20 and 28 g. About 40% of the tissue of the pancreas was removed in the experimental animals. Intact mice served as the control. The animals were decapitated at 10 a.m. on the 3rd, 4th, 5th, 7th, 16th, and 21st days after the operation, 6-8 animals at each time. All the animals were given intraperitoneal injections of [^{3}H]thymidine five times in the course of the 24-h period (at noon, 5 and 10 p.m., and 5 and 8 a.m.) in a dose of 0.25 μCi/g body weight. The specific activity of the isotope was 1.4 Ci/mmole. The pancreas was fixed in Bouin's fluid. Paraffin sections 4 μ thick were cut. The sections were coated with type M (NIKFI) emulsion and exposed for 45

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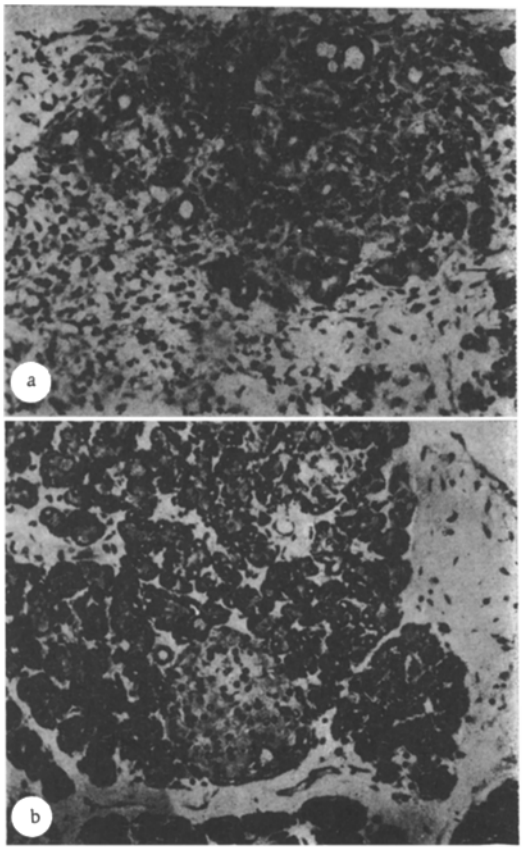

Fig. 1

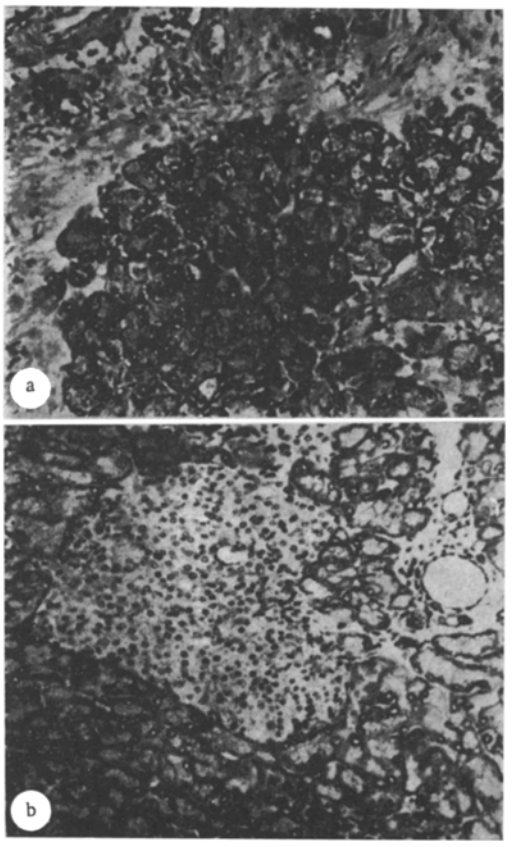

Fig. 2

Fig. 1. Pancreas on third day after operation. a) Zone of sharply defined reactive changes; b) first zone. Here and in Figs. 2 and 3: hematoxylineosin, 200×.

Fig. 2a. Pancreas on 21st day after operation. First zone. 2b) Pancreas on third day after operation. Second zone.

TABLE 1. Index (in %) of Labeled Acinar and Insular Nuclei in Three Zones of the Pancreas at Different Times after Operation on Mice Weighing 20 g (M ± m)

Group of animals	Zone of pancreas	Time after operation, days			
		3	4	7	21
		Islets			
Experimental	First	8,54±0,33	5,16±0,39	2,61±0,46	1,65±0,05
	Second	3,78±0,86	1,86±0,40	0,68±0,09	0,92±0,12
	Third	1,28±0,40	1,17±0,06	0,95±0,19	1,24±0,11
Control		1,51±0,21	1,51±0,21	1,43±0,31	1,15±0,19
		Acini			
Experimental	First	0,66±0,07	2,73±0,77	2,87±0,51	1,42±0,20
	Second	0,48±0,18	1,04±0,34	0,58±0,13	0,20±0,05
	Third	0,36±0,10	0,48±0,10	0,38±0,10	0,57±0,28
Control		0,48±0,14	0,48±0,14	0,31±0,06	0,33±0,11

days at −4°C. The autoradiographs were stained with Meyer's hematoxylin and eosin. The number of labeled nuclei in the experimental mice was counted in three zones of the gland separately. Altogether between 5000 and 12,000 nuclei of the acini and between 1500 and 2000 nuclei of the islet epithelium were examined in each case. Nuclei above which there were at least four grains of reduced silver were regarded as labeled. Virtually no background was present on these preparations. Besides labeled nuclei, the number of mitoses in the islets and acini also was counted. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The investigations revealed weak regenerative ability of the pancreas. After 21 days of the experiment no appreciable restoration of the weight of the organ had taken place. Several other workers likewise found no appreciable restoration of the weight of the pancreas after extensive resections [9-11].

The part of the pancreas adjacent to the zone of sharply defined reactive changes, but preserving a normal histological structure, was taken as the first zone. The zone of sharply defined reactive changes is illustrated in Fig. 1a. In the early period after the operation the acini and lobules of the gland in the first zone appeared to be separated because of edema of the interlobular and intralobular connective tissue (Fig. 1b). The acini and acinar cells preserved their typical histological structure. Pycnotic nuclei were rare. On the 21st day after the operation the acini and lobules of the pancreas no longer had the appearance of discontinuity as on the third day after the operation (Fig. 2a). In some places, however, discontinuity of the acini was observed, evidently on account of proliferation of connective tissue. In this zone the number of small cells in the acini and islets was increased compared with the intact control at nearly all times after the operation. It will be clear from Table 1 that the index of labeled nuclei in the islets was increased the most on the third day after the operation (control 1.51 ± 0.21%, experiment 8.54 ± 0.33%; $P < 0.001$). It then fell sharply and on the 21st day after the operation it was only 1.4 times greater than the control. The index of labeled nuclei in the acini increased rather later than in the islets, to reach a maximum on the 7th day after the operation (control 0.31 ±0.06%, experiment 2.78 ± 0.51%; $P < 0.001$). The number of labeled cells on the 21st day after the operation also was still increased almost fourfold.

The region just beyond the first zone relative to the site of injury was taken as the second zone. It also preserved its normal histological structure (Fig. 2b). The acini and lobules in this region of the gland were closely packed together. In this zone the number of labeled cells in the islets was increased on the third day after the operation. A small increase in the index of labeled nuclei was observed in the acini only on the fourth day after the operation.

In the third zone, the region furthest from the site of trauma (the duodenal loop), the number of labeled cells in the islets and acini was virtually the same as in the control.

In the older mice (weight 28 g) the same pattern was found in the distribution of labeled cells between the zones of the gland. However, the proliferative reaction was weaker than in the younger animals, especially in the epithelium of the acini. In the first zone, for instance, 5 days after the operation the number of labeled cells in the islets was 0.85 ± 0.10 in the control and 5.55 ± 1.80 in the experiment ($P = 0.021$). The corresponding figures for the second zone were: in the control 0.85 ± 0.10, in the experiment 1.96 ± 0.42 ($P = 0.025$). In the third zone the number of labeled cells did not differ significantly from the control values.

It was noted that the number of labeled cells in the islets of the mice of the two age groups was greater than in the acini. This finding agrees with the results of the writers' previous investigation, in which cell proliferation was studied in the intact pancreas [3]. Correlation was observed between the dynamics of the mitotic coefficient and the index of labeled nuclei in the young and aging mice, i.e., DNA synthesis was evidently directed toward preparation for mitosis and not polyploidization.

The proliferative activity of the pancreatic islets and acini in mice is thus significantly changed after partial resection of the organ. This depends not only on the time elapsing after the operation, but also on the distance of the region of the gland from the site of trauma.

Increased proliferative activity close to the wound surface in morphologically changed pancreatic tissue has also been observed by other workers who have studied regeneration of the pancreas [1, 2, 5, 6]. However, the absence of a proliferative reaction in regions of the organ remote from the site of trauma has been reported only for the acini in a paper by Zelenina [2], who used hybrid mice for her investigations, as in the present series.

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CHARACTERISTICS OF THE ESTROGEN RECEPTOR SYSTEM OF THE GUINEA PIG UTERUS

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The physicochemical parameters of estradiol–receptor (E_2–R) interaction in the cytosol of guinea pig uterus (the velocity constants of association and dissocation, the half-life of the E_2–R complex, the change in free binding energy) were studied and the content of receptors in the cell calculated. With different degrees of approximation to a state of equilibrium, for most steroids the percentage of affinity for the test system remained unchanged, indicating that equilibrium had been reached in these cases. The affinity of the steroids for the R system analyzed was determined by integrity of the 3- and 17 β-hydroxyl groups and by the somewhat greater importance of the third phenolic hydroxyl group of the steroid molecule.

KEY WORDS: uterus; steroid–receptor interaction; specificity; estrogens.

This investigation is a continuation of a study of the estradiol–receptor (E_2–R) system of the guinea pig uterus started previously [1].

Kinetic and thermodynamic parameters and also the specificity of interaction of a number of steroids with the receptor system of the uterus were studied.

EXPERIMENTAL METHOD

Uteri of sexually immature guinea pigs weighing 140-200 g were removed and treated by the usual method [1] with dilution of the tissue with buffer 1:10 for homogenization. The cytosol was kept at −30°C. The residue was used for DNA determination [3]. 2,4,6,7-Estradiol-17β-^{3}H (E_2-17β-^{3}H) (specific activity 100 Ci/mmole, Amersham) and unlabeled compounds, whose names are given in Table 1, were used in the experiments. The velocity constants of dissocation (k_{-1}) and association (k_{+1}) and the half-life of the complex ($T_{1/2}$) were determined by the usual methods [8]. To determine the equilibrium association constant (K_{as}) of estradiol with the isolated system the principle of saturation analysis was used [4]. The range of concentrations of labeled estradiol was 0.05-0.75 pM. The free and bound fractions were separated by means of a suspension of charcoal (0.25% suspension in buffer containing 0.1% gelatin). To allow for nonspecific binding, a hundred-fold excess of unlabeled estradiol was used [5]. K_{as} and the concentrations of binding sites were calculated by Scatchard's

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