corticosterone level in CBA mice was combined with a high lipid content in the adrenal cortex, whereas in AKR and C57BL/6 mice the lipid content in the adrenal cortex was low. AKR mice are known to be distinguished by hypofunction of the adrenal cortex, with which the characteristic preleukemic changes in the thymus tissue are associated [9]. In the present experiments the CBA mice did not react to sex antigen, whereas in C57BL/6 and AKR mice a well-marked reaction to this antigen was observed, in agreement with data obtained by other workers [8].

On the basis of comparison of the morphological and functional data it can thus be postulated that adrenocortical function and the blood plasma corticosterone level in inbred mice may affect the thickness of the cortical layer of the thymus, the morphology of the spleen, and the intensity of the immunologic reaction induced by weak transplantation antigen. Adrenalectomy on CBA mice led to a sharp increase in width of the cortex of the thymus, to the appearance of many lymphatic follicles with wide reactive centers in the spleen, and to the development of an immunologic reaction to the skin graft sex antigen.

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LOCALIZATION OF TAGGED CELLS IN THE ISLETS, ACINI, AND PERI-INSULAR ZONE OF THE MOUSE PANCREAS AFTER RESECTION

V. P. Krasnov

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[^{3}H]Thymidine was injected five times in the course of the 24-h period into CBA × C57BL/6 hybrid mice in which about 40% of the tissue of the pancreas has been resected. The number of tagged cells in the islets and acini did not change with an increase in the time elapsing after the last injection of thymidine (5-18 days). The localization of the tagged cells in the pancreatic islets in the later stages did not differ significantly from that observed 2 h after injection of the isotope into the animals. On examination of medium-sized and large islets as far removed as possible from the site of injury, a very similar number of tagged cells per unit area was found in the central, middle, and peripheral zones of the islets 2 h and 18 days after the last injection of the isotope.

KEY WORDS: *mouse pancreas; localization of tagged cells; peri-insular zone.*

The problem of the sources of formation of the pancreatic islets in intact animals and under experimental conditions still remains a matter for debate. Many workers consider that the formation of the cell population of the islets takes place on account of external sources: 1) by transformation of exocrine cells from the terminal portions [1, 2, 4-7, 9, 12, 13]; 2) from cells of the ducts [3, 10, 11, 14]; 3) from special precursor cells, also belonging to

Department of Anatomy, Moscow Medical Stomatologic Institute.

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the duct system [8]. There are considerable technical difficulties in the way of proving which of these possible ways of formation, growth, and regeneration of the islets is correct. Investigators have most frequently been compelled to interpret the process of conversion of one type of cells into another by comparing static pictures. This method, as we know, must be used with great caution. Only in one study [8] was an attempt made to trace the localization of cells labeled with [^{3}H]thymidine in the islets and ducts of intact mice at different times after injection of the isotope.

The object of this investigation was to detect any possible migration of cells after resection in areas of the pancreas located at different distances from the site of injury. For this purpose the number of tagged cells was compared at different times after injection of [^{3}H]thymidine in the islets, the acinar cells of the peri-insular zone, and the remainder of the acini. At each chosen time the localization of the labeled cells in the islets was noted.

EXPERIMENTAL METHODS

Experiments were carried out on (CBA × C57BL/6) hybrid mice of two age groups (mean weight 20 and 28 g). About 40% of the pancreatic tissue was removed from the mice. [^{3}H]-thymidine was injected five times in the course of the 24-h period into the animals after the operation. [^{3}H]Thymidine (USSR) with a specific activity of 1.4 Ci/mmole was used in the experiments in a dose of 0.25 μCi/g body weight. The animals weighing 20 g were divided into four subgroups. In subgroups 1 and 2 [^{3}H]thymidine was injected on the 2nd day after the operation. The animals of subgroup 1 were killed 2 h after the last injection of the isotope, those of subgroup 2 five days later (i.e., on the 7th day after the operation). [^{3}H]Thymidine was injected into the animals of subgroups 3 and 4 on the 3rd day after the operation, and the animals were killed 2 h (subgroup 3) and 18 days (subgroup 4) after the last injection of thymidine. The mice weighing 28 g were divided into two groups. [^{3}H]Thymidine was injected into these animals on the 4th day after the operation. One subgroup was killed 2 h after the last injection of the isotope, the other 12 days later (on the 16th day after the operation).

The pancreas was fixed in Bouin's fluid. Two pieces of the gland were taken from each mouse. Paraffin sections 4 μ thick were cut. The sections were coated with type M (NIKFI) emulsion and exposed for 45 days at −4°C. The autoradiographs were stained with Mayer's hematoxylin–eosin. The number of labeled cells was counted in the region farthest from the site of injury (in the loop of the duodenum) and in an area close to the wound, where the gland tissue preserved its typical histological structure. When the tagged cells were counted in the islets, their localization was noted by measuring the shortest distance between the cell and the edge of the islet. The number of tagged nuclei of acinar cells directly bordering on the islets (peri-insular cells) and in the remainder of the acini was counted separately.

TABLE 1. Index of Labeled Nuclei (%) in Islets, Acini, and Peri-Insular Zone of Mice Weighing 20 and 28 g at Different Times after Injection of [^{3}H]Thymidine (M±m)

Subgroup of animals	Time of injection of [^{3}H] thymidine (day after operation)	Time elapsing after last injection of isotope and before sacrifice	Location of region of gland relative to site of trauma	Islets	Peri-insular zone	Acini
Mice weighing 20 g						
1	2-nd	2 h	Near	3,78±0,86	3,74±0,52	0,48±0,18
			Distant	1,23±0,40	2,62±0,65	0,36±0,10
2	2- "	5 days	Near	4,78±1,25	1.42±0,58	0,32±0,18
			Distant	1,82±0,30	2.89±1,78	0,56±0,20
3	3-rd	2 h	Near	1,86±0,40	2.93±0,88	1.04±0,34
			Distant	1,17±0,06	3.53±1,08	0,48±0,10
4	3- "	18 days	Near	2,96±0,47	4,04±1,27	0,70±0,25
			Distant	1,62±0,32	1.86±1,09	0,68±0.41
Mice weighing 28 g						
1	4- th	2 h	Near	1,96±0,42	6,25±4,40	0,26±0,17
			Distant	1,11±0,40	0.77±0,39	0,18±0,02
2	4- th	12 days	Near	1,35±0,16	2,12±1,19	0.37±0,08
			Distant	0,80±0,24	0,97±0,58	0,06±0,01

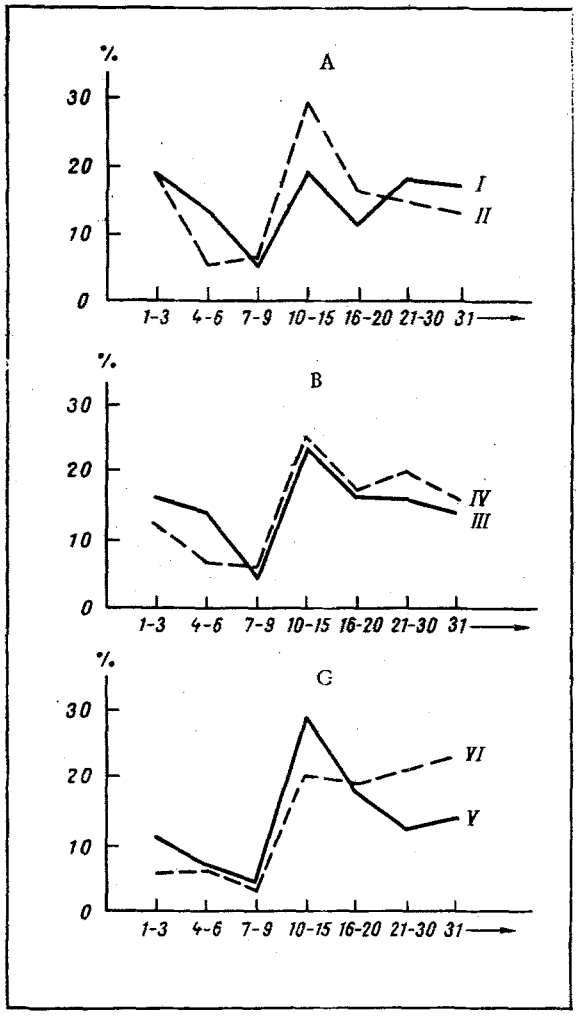

Fig. 1

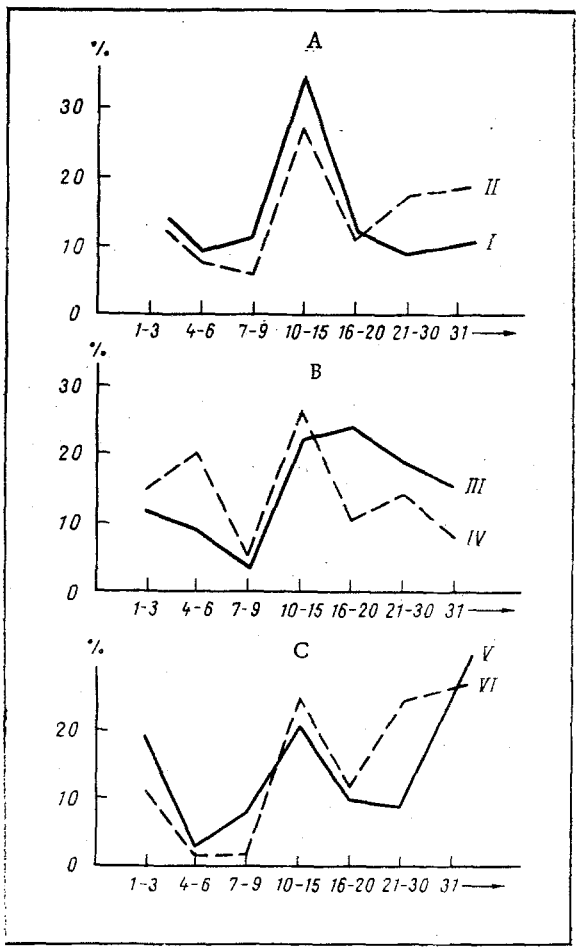

Fig. 2

Fig. 1. Localization of label in islets situated near to site of injury, 2 h (I, III, V), 5 days (II), 12 days (VI), and 18 days (IV) after last injection of [^{3}H]-thymidine. A) Mice weighing 20 g; [^{3}H]thymidine injected on 2nd day after operation; B) mice weighing 20 g; [^{3}H]thymidine injected on 3rd day after operation; C) mice weighing 28 g; [^{3}H]thymidine injected on 4th day after operation. Abscissa) shortest distance between tagged cell and edge of islet in ocular micrometer units (one division = 0.8 μ); ordinate) number of tagged nuclei in a given location (in % of total number of tagged nuclei).

Fig. 2. Localization of label in islets in regions of gland distant from site of trauma, 2 h (I, III, V), 5 (II), 12 (VI), and 18 days (IV) after last injection of [^{3}H]thymidine. Legend as in Fig. 1.

Altogether in each case between 5000 and 12,000 nuclei of acinar cells, 1500-2000 nuclei of the epithelium of the islets, and an average of 150 nuclei of acinar cells of the peri-insular zone were examined.

Nuclei were regarded as labeled if at least 4 grains of reduced silver were present above them. Virtually no background radioactivity was present. The numerical results were subjected to statistical analysis by the Student—Fisher method.

EXPERIMENTAL RESULTS

The number of tagged cells in the acini, peri-insular zone, and islets is given in Table 1 for regions located at different distances from the site of trauma. In the pancreatectomized mice of all subgroups the index of labeled nuclei in the acinar epithelium, irrespective of the distance from the site of the operation, showed virtually no change with an increase in the interval between the last injection of [^{3}H]thymidine and sacrifice of the animals from

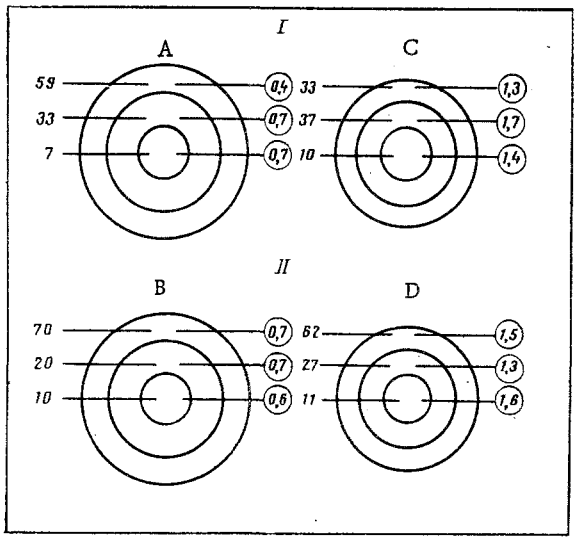

Fig. 3. Distribution of tagged cells among zones of islet (outer, middle, and central). Region of gland distance from site of injury. A, B) Large islets (100 cells and more per section); C, D) middle-sized islets (50-80 cells per section). I and II) 2 h and 18 days respectively after last injection of [^{3}H]thymidine. Numbers on left indicate distribution of tagged nuclei, in %, among zones of islet; numbers on right (circled) denote percentage of tagged cells per conventional unit area of zone.

5 to 18 days. No significant differences likewise were found in the index of labeled nuclei in the islets with an increase in the time between the last injection of the isotope and sacrifice. However, in young animals in all cases the percentage of labeled nuclei was a little higher with an increase in the time before sacrifice. The tendency observed was possibly not accidental and the number of labeled cells may in fact have increased in the islets. Such an increase could be due to several causes: 1) repeated division of the tagged cells, 2) conversion of the labeled acinar cells of the peri-insular zone into islet cells, 3) conversion of precursor cells located in the ducts and their onward migration. The decrease in the percentage of labeled cells in the peri-insular zone may be confirmation of the second of these causes. In fact, in the mice of subgroup 2 close to the site of injury and in the mice of subgroup 4, at a distance from it, a decrease in the index of labeled nuclei was observed in the acinar cells of the peri-insular zone. It was statistically significant ($P = 0.01$). However, the preservation of the original localization of the tagged cells in the islets is evidence against any transmission of labeled exocrine cells of the peri-insular zone into islet cells. Satisfactory coincidence between the two curves reflecting the frequency of discovery of tagged cells at different distances from the edge of the islet 2 h and 5 days after injection of the label can be seen in Fig. 1A. In the other cases also, the curves reflecting the localization of tagged cells in the islets 5, 12, and 18 days after injection of the isotope also were similar to the curves reflecting their localization immediately after injection of the label (Fig. 1B; Fig. 2A, C). Only in the mice of subgroup 4 remote from the site of trauma was the curve obtained 18 days after injection of [^{3}H]thymidine not quite typical (Fig. 2B): The curve had two peaks. One of them indicated that tagged cells were more often located near to the edge of the islet. It was therefore decided to subject the data on the localization of tagged cells in the mice of subgroup 4 to further analysis for large and medium-sized islets. It will be clear from Fig. 3 that more than half of all the tagged cells lay in the outer zone of the islet. This predominance depends entirely on the fact that the area of this zone is much greater than the area of the middle and central zones. When the number of labeled cells per unit area was calculated it was about the same in all zones of the islet. The localization of the tagged cells 18 days after the last injection of [^{3}H]thymidine showed no significant change. This preservation of the original localization of the tagged cells is also evidence against intensive conversion and onward migration of the cells from the duct system into the islets.

It can thus be concluded from this investigation that after resection of the pancreas tagged cells of the islets and acini did not divide a second time in the course of the experiment and remained in the place where they incorporated the label. Increased formation of the cell population of the islets on account of other sources (exocrine cells or special precursor cells) evidently was not observed either.

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ORIGIN OF THE KUPFFER MACROPHAGES IN THE REGENERATING LIVER

D. N. Mayanskii, V. I. Shcherbakov, and Yu. M. Mirokhanov

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[^{3}H]Thymidine was injected intraperitoneally into male Wistar rats in a dose of 5 μCi/g, and two thirds of the liver was resected 1 h later. Control animals underwent a mock operation. Indices of labeled nuclei (ILN) of the hepatocytes were moderately increased 3 h after partial hepatectomy, the increase still continued for 48 h, but was replaced by a decrease 48 h after the operation. ILN of the Kupffer cells was 10 times higher 3 h after the operation than in the control, it reached a maximum 9 h after the operation, and then fell, although still remaining higher than in the control. It is concluded that Kupffer cells enter the liver from the bone marrow.

KEY WORDS: *hepatectomy; Kupffer cells; regeneration of the liver; autoradiographic labeling.*

The writers showed previously that 3 h after partial hepatectomy in rats the relative number of Kupffer cells (KC) in the liver rises significantly to reach a maximum 9 h after the operation (45% compared with 35% in the control; $P < 0.01$). Later, 24 h and, in particular, 36 h after partial hepatectomy the number falls, and 72 h after the operation shows a tendency to rise again [3]. In connection with this observation the question arises of the origin of the additional KC pool in the liver of hepatectomized rats. It has first to be discovered whether they accumulate on account of transformation of some of the mesenchymal liver cells into Kupffer cells *in situ* or through the arrival of precursors of Kupffer macrophages from extrahepatic sources.

To study this problem the approach described in the literature to the study of the cytogenetics of peritoneal [9], pulmonary [8], neuroglial [7], and other classes of macrophages, combined into the single system of mononuclear phagocytes [5], was used. The approach is based on the fact that 1 h before stimulation of the macrophagal reaction the animal is given an injection of [^{3}H]thymidine, on the grounds that during this period the tritium label will be incorporated into all DNA-synthesizing cells, including precursors of cells of the histiomonocytic series. Differentiated forms of tissue macrophages, including KC, are known virtually not to synthesize DNA and not to incorporate [^{3}H]thymidine [6, 9]. Hence the accumulation of labeled KC in a focus of stimulation of a macrophage reaction, in the present case in the regenerating liver, would indicate their arrival from an external source in the form of DNA-synthesizing precursors.

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