AUTORADIOGRAPHIC INVESTIGATION OF THE LEVEL OF PROLIFERATION IN PANCREATIC ACINAR AND ISLET CELLS OF INTACT MICE

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In the course of the 24 hours CBA × C57B1/6 hybrid mice received 5 injections of thymidine-H^3. This method revealed a diurnal fraction of cells synthesizing DNA and which in mice weighing 20 g amounted to 1.37% in the pancreatic islets and 0.66% in the acini, while in mice weighing 28 g the proportions were 0.79 and 0.32%, respectively. The labeling index was higher in acinar cells adjacent to the islets than in the remaining cells.

The proliferative activity of the epithelium of the pancreatic islets has been insufficiently studied. The view was held for a long time that mitosis does not take place in the islets of Langerhans in adult mammals. However, the first investigations using thymidine-H^3 showed that a certain proportion of cells in the islets of Langerhans is in the period of DNA synthesis. In investigations in which a single injection of thymidine-H^3 was given the frequency of its incorporation into the cells of the islets and acini was found to be equal, about 1% [2, 7-9]. Determination of the level of mitotic activity in later investigations [2, 3, 4] also failed to reveal any significant differences between the islets and acini. More recently work has been published in which a higher percentage of labeled cells was found in the islets than in the acini by a technique of prolonged administration of thymidine-H^3 [1, 6].

The object of the present investigation was to study the proliferative activity of the epithelium of the islets and acini of the mouse pancreas by repeated injections of thymidine-H^3 for a period of 24 h. It was

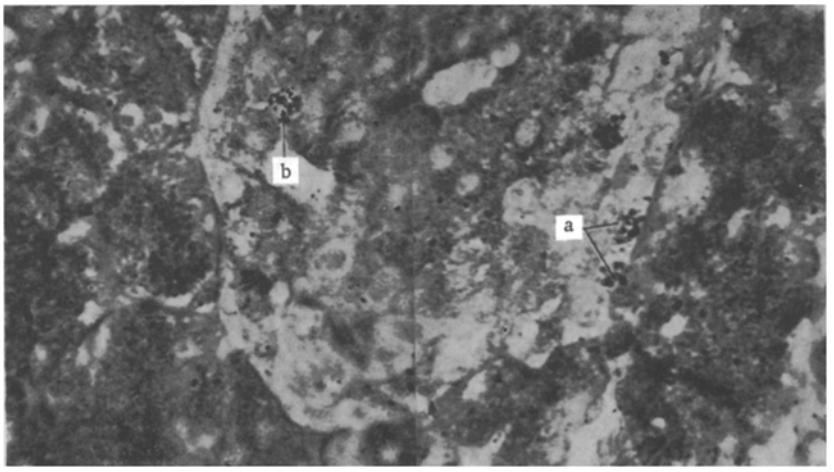

Fig. 1. Labeled α-cells (a) and β-cells (b) in an islet of Langerhans (aldehyde-fuchsin by Gabe–Dyban method, counterstained by Halmi's method, gilded).

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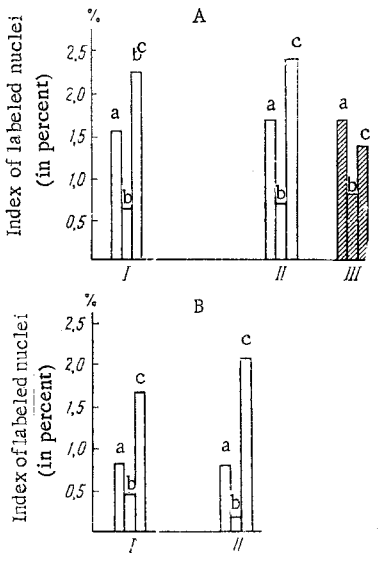

Fig. 2. Index of labeled nuclei (in percent): A) in mice with an initial body weight of 20 g: I) subgroup 1; II) subgroup 3 (2 h after last injection of thymidine-H^3); III) subgroups 2 (18 days after last injection of isotope); B) in mice with initial body weight of 28 g; I) subgroup 1; II) subgroup 2 (2 h after last injection of isotope; a) islets; b) acini; c) acinar cells bordering in islets.

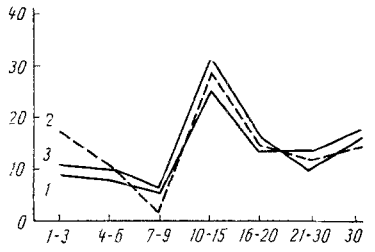

Fig. 3. Localization of labeled cells in islets of mice with initial body weight of 20 g: I) subgroup 1; II) subgroup 2; III) subgroup 3. Abscissa, shortest distance between labeled cells and border of islet in ocular micrometer units (1 division = 0.8 μ); ordinate, number of labeled nuclei with a particular localization (in percent of total number of labeled nuclei).

considered that the diurnal fraction of calls taking part in proliferation and revealed by this method could serve as a true index of proliferative activity, whereas the index of labeled nuclei determined after a single injection of thymidine-H^3 gives only the size of the fraction of cells existing simultaneously in the period of DNA synthesis (the longer the S-period, the relatively larger this fraction). In addition, the localization of labeled cells relative to the boundary of the islets was examined.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA × C57B1/6 hybrid mice of 2 age groups with mean body weights of 20 and 28 g. All the experimental animals received an intraperitoneal injection of thymidine-H^3 with a specific activity of 1.4 Ci/mmole 5 times in the course of the 24 hours (at noon, 5 and 10 p.m., and 3 and 8 a.m.) at the rate of 0.25 μCi/g body weight. The mice weighing 20 g were divided into 3 subgroups. The animals of subgroups 1 and 2 were injected simultaneously with thymidine-H^3, but the 12 mice constituting subgroup 1 were killed 2 h after the last injection, whereas the 5 mice of subgroup 2 were killed 18 days after that injection. Simultaneously with the mice of group 2, animals of subgroup 3 (7 mice) were killed: these mice received thymidine-H^3 by the same scheme of injections during the 24 h before sacrifice. Subgroup 3 was thus essentially analogous to subgroup 1, but by this time the mice were 18 days older. In the mice of age group 2 the experiment was also repeated, but at an interval of 12 days, and in both subgroups the mice (six and five animals, respectively) were killed 2 h after the last injection of thymidine-H^3.

The pancreas was fixed in Bouin's fluid and paraffin sections, 4 μ in thickness, were prepared. The sections were coated with diluted (1 : 3) type M emulsion and exposed for 45 days at −4°C. The autoradiographs were stained with Mayer's hematoxylin and eosin and with aldehyde-fuchsin by the Gabe−Dyban method and counterstained by Halmi's method in order to demonstrate the α- and β-cells of the islets of Langerhans separately. In the last case, the sections were gilded to preserve the label (Fig. 1) [5].

Most of the counting was carried out on specimens obtained with hematoxylin and eosin. The index of labeled nuclei was determined in the epithelium of the acini and islets. The localization of the labeled cells was determined in the islets by measuring the shortest distance between the labeled cell and the border of the islet. In addition, when the number of labeled cells was counted in the islets, the nuclei of the acinar cells directly bordering the islets was counted, and the index of labeled nuclei determined for these boundary acinar cells. Altogether in each case from 5,000 to 12,00 nuclei of the acini, 1,500-2,000 nuclei of the islet epithelium, and on the average 150 nuclei of acinar cells bordering the islets were examined. Nuclei were regarded as labeled if they had 4 grains of reduced silver or more over them. The numerical results were subjected to statistical analysis by the Fisher−Student method.

EXPERIMENTAL RESULTS

In all three subgroups of young mice a relatively high index of labeled nuclei was found for the islet cells: in group 1) 1.54 ± 0.15%,

2) 1.66 ± 0.34%, and 3) 1.15 ± 0.1%. The labeling index in the acinar cells was between one-third and one-half lower than in the islets: 0.63 ± 0.11, 0.80 ± 0.44, and 0.69 ± 0.44%, respectively (Fig. 2a). Individual variations for the epithelium of the islets were less marked than for the epithelium of the acini (see the relative magnitude of the error of the mean).

Examination of the specimens showed that labeled nuclei were relatively frequent in the acinar cells bordering on the islets. Special counts showed that labeled nuclei were more than 3 times more frequent among acinar cells bordering on the islets than in the rest of the acini. This pattern was found in all groups of the experiment. On the average for the young mice the index of labeled nuclei in the acinar epithelium was 0.66%, while in the cells bordering on the islets it was 2.3% (P = 0.036). The number of labeled nuclei in the islets and also in the acini was very similar in all three subgroups. The similarity between the indices for subgroups 1 and 3 points to the absence of any significant changes in the level of proliferation in the mice of that particular group during the 18 days of the experiment. (The weight of the animals during this period increased on the average by 3.5 g, and the weight of the pancreas from 233 to 244 mg.) For the statistical analysis it was therefore possible to combine the data for these two subgroups. (In both cases the mice were killed 2 h after the last injection.) The mean index of labeled nuclei was 0.66% for the epithelium of the acini and 1.34% for the epithelium of the islets (P = 0.002). The similarity between the results for subgroups 1 and 2 is explained by the fact that most cells labeled and dividing at the beginning of the experiment did not divide again during the 18 days that it lasted. Maintenance of the localization of the labeled nuclei in the islets during this period is shown by the results of determination of the position of the labeled nuclei with respect to the border of the islet. Curves showing the frequency of discovery of labeled nuclei in different locations are shown in Fig. 3. These curves were very similar for all 3 groups.

In the older mice (28 g), just as in the young animals, the number of labeled cells in the islets was greater than in the acini (Fig. 2b). The index of labeled nuclei both in the islets and in the acini showed no significant change in animals sacrificed at the two different times separated by an interval of 12 days. The mean index of labeling in the animals of this group was 0.77% in the islets and 0.21% in the acini. The difference between the islets and acini was significant (P = 0.000).

Comparison of the results for the islets and acini of the animals of the two age groups shows that the number of labeled cells in both islets and acini falls appreciably with age (1.34 and 0.77%, respectively, in the islets, P = 0.012; 0.66 and 0.21% in the acini, P = 0.021).

The results of these experiments are in agreement with those obtained by Cameron [6] who determined the renewal time of the cells from the slope of the curve of increase in the index of labeled nuclei during continuous administration of thymidine-H^3 with the drinking water. According to his observations, in mice which had ceased to grow the period of renewal for the epithelium of the pancreatic acini was 520 days, and for the epithelium of the islets 250 days. This corresponds to a diurnal fraction of DNA-synthesizing cells of 0.2 and 0.4%. Since in the present experiments the mice were younger than those used by Cameron, the indices were higher.

The method of repeated injection of thymidine-H^3 thus revealed a higher level of proliferative activity in the epithelium of the islets than in the epithelium of the acini, which could not be discovered by any other method. This fact suggests that renewal of the islet cells during physiological regeneration takes place through division of α- and β-cells.

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