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MORPHOLOGICAL CHANGES IN THE PANCREAS DURING THE DEVELOPMENT OF EXPERIMENTAL DIABETES

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A number of studies of alloxan diabetes have been made [1-4, 7-12, 14]. Nevertheless, until now, the origin of the new islets have not been identified, and nothing is known of the quantitative relationship between the alpha- and beta-cells, nor of how they become transformed to the islets of Langerhans. Also insufficient work has been done on the changes occurring in the exocrine portion of the gland in this disease.

The object of the present work has been to study the histological changes of the rat pancreas during the gradual development of diabetes induced by daily subcutaneous injections of small doses (5 mg/100 g weight) of a 5% aqueous alloxan solution, while the blood sugar was controlled by the Hagedorn-Hensen method.

EXPERIMENTAL METHODS

The work was carried out on 62 white male rats weighing 180-260 g; 15 animals which did not receive alloxan served as controls. The experiment lasted from 1 to 33 days. All the rats were maintained on precisely the same diet. During the whole of the experiment observations were made on the external appearance, weight and the extent to which the animals could be excited by food. They were killed by decapitation at various times during the development of the disease.

The material was fixed in Bouin's fluid and in Zenker-formal. Paraffin sections were stained by the usual methods, and the islet cells were revealed by Gomori's method [13]. A count of the alpha- and beta-cells was made on 10 islets of Langerhans in each animal. The figures were treated statistically.

EXPERIMENTAL RESULTS

An analysis of the histological changes of the pancreas and of the blood sugar curves revealed the variation in their sensitivity to a given dose of alloxan, and on this basis the animals could be divided into three groups.

The first consisted of 24 rats in which during the whole of the experiment the blood sugar concentration remained within the normal limits of 85-105 mg%. * In the second group there were 18 animals in which the blood sugar was raised to 125-163 mg%, while the third group consisted of five animals with an even higher blood sugar level of 189-264 mg%. In all the animals the increase in blood sugar did not occur at the same time after the injection of alloxan, which was given on the 4th, 10th, 17th, 23rd, and 28th days. However, as a rule the nature and intensity of the changes in the islets of Langerhans represented a functional index of the condition of the blood and of the animal's general condition, independently of how long it had been ill.

In the rats of the first group, despite the normal blood sugar level, there was a moderate pancreatic hyperemia. Solitary degenerating beta-cells were found in the islets of Langerhans. Some of them were increased in size, in others no nuclei were visible, and the cytoplasm showed no granularity or cell outline. The amount of DNA in the nuclei of these cells was less than in the unchanged cells. Besides the necrosis of the beta-cells, like other authors

* In healthy rats the blood sugar has an average value of 95 ± 10 mg%.

[2, 3, 4, 9, 10, 12, 14] we found that occasionally they multiplied mitotically. We could observe no special change in the alpha-cells. The ratio of the number of cells of the islets of this group of rats was not normal; there were $91 \pm 2.3\%$ beta-cells, and $9 \pm 2.3\%$ alpha-cells.

In the second group the hyperemia of the pancreas was more marked. The destructive changes in the epithelium of the islets of Langerhans resembled those found in the first group, but there were more beta-cells. It must be emphasized that as the beta-cells fell to $71 \pm 3.3\%$, the number of alpha-cells increased to $29 \pm 3.3\%$. The latter developed at the periphery of the individual islets and were formed from protoplasmic structures having closely packed nuclei, and rather indefinite cell borders (Faller in 1955, described them as a "plasmodium"). The same thing was found in preparations stained with Heidenhain's iron hematoxylin. During the development of the alpha-cells from the "plasmodium" cytoplasm around the individual nuclei increased; around them also appeared cell borders, and a cytoplasmic granularity resembling that of the alpha-cells (Fig. 1). The micrograph shows that the "plasmodium" lies near the interstitial portion, and occupies a considerable part of the islet. In control animals, these structures are also found lying close to the islets of acini, but they are smaller in size than in the affected rats. Probably the formation of the "plasmodium" both in health and in diabetes takes place through the multiplication of cells of the interstitial parts of the gland which are but slightly differentiated.

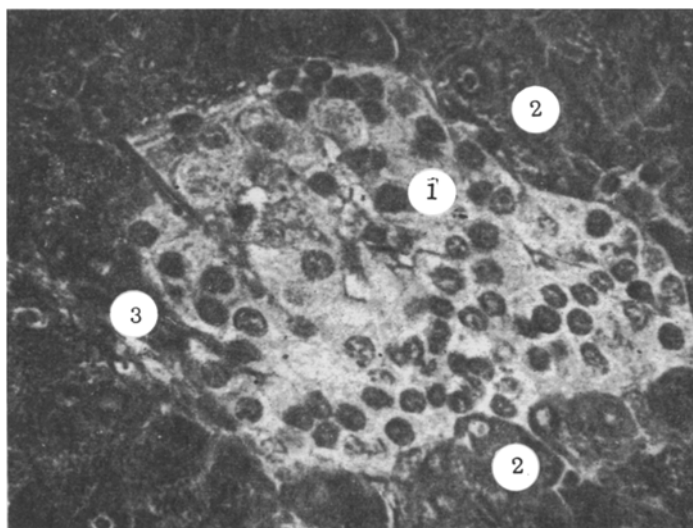


Fig. 1. Pancreatic gland of white rat 11 days after the injection of alloxan. Blood sugar $125 \text{ mg}\%$. 1) Islet of Langerhans; 2) acini of "peri-insular zone," filled with zymogen granules; 3) marked increase in the nuclei of the interstitial cells. Fixation in Bouin, Masson's stain. Magnification $800 \times$.

In animals of the third group, in the islets of Langerhans there was a preponderance of alpha-cells, which represented $65 \pm 6.6\%$ of the total, whereas the beta-cells constituted only $35 \pm 6.6\%$. There were also islets which contained only alpha-cells (Fig. 2); no "plasmodia" were to be seen.

In the exocrine part of the gland, in the sick rats the binucleate acinous cells in the "peri-insular" zone were more numerous than in the control. Their increase in number may have been due to an increased mitotic activity of the acinous cells as Duff [11] found in rats with alloxan diabetes. In the same zone of the islet, sometimes acinous cells with a greatly altered granular cytoplasm were observed. Here the cytoplasm of the apical portion lost its characteristic basophilia and zymogen granules, whereas the newly formed granularity stained as it did in the beta-cells. The basal portion of these cells still retained a marked basophilia, and the nuclei were unaltered. These changes are one of the indications of regeneration of the islet cells from the exocrine part. Some authors [3, 5, 6] observed the same kind of transformation of the acinous cells into islet cells under other experimental conditions.

It should be noted that as the diabetes develops, the size of the islets of Langerhans changes. In healthy rats chiefly large and medium sized islets are found and there are far fewer small ones; in diabetic rats, small and

medium sized islets were more common, and the large islets were encountered only exceptionally. Apparently the small islets are newly formed, and develop from the interstitial portions because they are connected to each other.

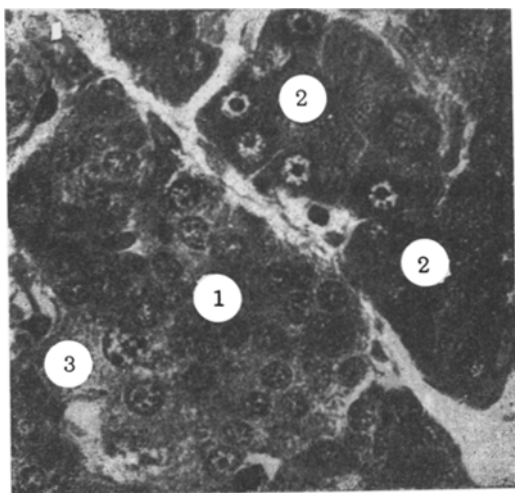


Fig. 2. Pancreas of white rat 13 days after the injection of alloxan. Blood sugar 204 mg%. 1) The islets of Langerhans consist of alpha-cells only; 2) acini of the "peri-insular zone," filled with zymogen granules; 3) two acinus cells greatly enlarged before division. Fixation in Bouin. Gomori's stain. Magnification 800 x.

The use of small doses of alloxan enabled us to observe histochemical changes in the pancreas for a long time and to establish that there was a direct relationship between the nature and intensity of the morphological changes and the degree of disturbance of carbohydrate metabolism. With increase of hyperglycemia, the process of necrosis of the beta-cells was accelerated. In addition, there was some regeneration of the beta-cells through mitosis and transformation of some of the acinous cells. At the same time there was also an increase in the size of the "plasmodium," which gave rise to new alpha-cells. This process appears to be one of the compensatory mechanisms.

SUMMARY

Repeated administration of small doses of alloxan (5 mg/100 g of body weight) caused diabetes to develop gradually in albino rats. A direct relationship could then be observed between the degree of disturbance of the carbohydrate metabolism and the extent of the morphological changes in the pancreas; as the blood sugar concentration in the islets of Langerhans rose, the death rate of the beta-cells also rose, and new gamma-cells were formed which developed from the "plasmodium." Most of the medium sized and small islets were found in diabetic rats. Changes were found in the acinous cells of the "peri-insular zones," indicating their possible transformation into islets.

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