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Effect of Administration of Parotin and Excision of Parotid Salivary Glands on the Morphological Picture of Islets of Langerhans in the Pancreas of the White Rat

[With Plates III—IV]

Using morphological methods it was found that a connection exists between the parotid salivary glands and the istlets of Langerhans in the pancreas. Administration of parotin caused a reduction in the number of A cells (secreting glucagon), with simultaneous reduction in the dimensions of their nuclei and increase in the number of B cells. The action of parotin would therefore be synergic to glucagon and antagonistic in relation to insulin. Removal by operation of the salivary glands caused an increase in the number of A cells and increase in the dimensions of their nuclei, which also confirms the hyperglycemic action of the parotid salivary glands. Excision of the parotid salivary glands after 35 days ceases to affect the morphological picture of the islets of Langerhans. It was assumed that the function of the excised salivary glands was taken over by the submandibular salivary glands.

I. INTRODUCTION

Studies have appeared in literature since 1928 (Budges — cited after Sawicki, 1960), on the endocrine function of the salivary glands.

In the years following the above date (1929—1941) Japanese research workers (Ogata, 1955; Ito et al., 1954; 1955) published several papers describing how they confirmed the existence of the endocrine function of salivary glands by the use of physiological, biochemical and histochemical methods. In 1944 these scientists obtained from salivary glands a biologically active substance, of the nature of protein, which was held to be the specific hormone of the parotid glands and given the name of parotin.

Many authors have demonstrated the existence of correlation between the salivary glands and other endocrines (Ito, 1960; Lacassagne, 1940; Parhon et el., 1957; Fleming, 1960; Raynaud, 1954a, b; Shofer & Muhler, 1960; Sawicki, 1960; Dzierżykray-Rogalska & Gutsze, 1963; Dzierżykray-Rogalska, 1963).

The problem of the role of salivary glands in carbohydrate metabolism has been examined by many authors for a considerable period: Reals, 1890; Ogata, 1955; Dionesov, 1952; Hill et al., 1955; Parhon et al., 1956; Parhon et al., 1957; Dehaume, 1958; Grandos, 1951; Birnkrant & Shapiro, 1942.

Reale (1890), Ogata (1955) found that the salivary glands exhibit hypoglycemic activity, and Dionesov (1952) — that the action of the parotid glands in carbohydrate metabolism is synergic with the action of insulin.

On the other hand authors such as Birnkrant & Shapiro (1942), Grandos (1951), Parhon et al. (1956) report the hyperglycemic function of parotin. Hill, Matt & Pearlman (1955) obtained a hyperglycemic factor from perotid salivary glands by extraction. This extract caused hyperglycemia when given to rats. Similar experiments made on rabbits by Parhon et al. (1956) confirmed the hyperglycemic function of the salivary glands. It would therefore appear from these investigations that extract from parotid glands has an antagonistic action in relation to insulin (inhibits hypoglycemic function — Parhon et al. 1956).

In view of the differences between opinions held by authors engaged in research on the effect of parotid salivary glands on sugar metabolism and the islets of Langerhans in the pancreas, we were interested in examining the behaviour of cells A and B after parotin [1]) had been administered and after the excision of the parotid salivary glands.

II. MATERIAL AND METHODS

Fifty young, sexually mature, male white rats, weighing about 200 g, were used for the xperiments. The animals were kept on a standard granulated diet [2]).

The animals were divided into three groups:

Group *I* — control animals

Group *II* — animals injected with parotin

Group *III* — animals from which the parotid salivary glands had been excised by operation, and which were later dissected at different intervals of time.

Group *I* (control) included 3 sub-groups:

1. animals left untouched — 3 rats
2. animals which were given an intramuscular injection of normal saline solution — 0.1 ml/100 g of body weight — 3 rats
3. animals on which a "blind" operation was performed, i.e. without removal of glands — 4 rats

Group *II* included:

1. animals which were given 0.1 mg of parotin per 100 g of body weight over a period of 7 days — 10 rats
2. animals, which were given 0.2 mg of parotin per 100 g of body weight over a period of 7 days — 10 rats

Group *III* included:

1. animals dissected 7 days after excision of the parotid glands — 10 rats
2. animals dissected 21 „ „ „ „ „ „ „ — 5 rats
3. animals dissected 35 „ „ „ „ „ „ „ — 5 rats

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[1]) The author has pleasure in expressing her gratitude to the Teikoku Mfg. Co., Tokio, for supplying free of charge the parotin used in the preparation of this study.

[2]) Granulated feed for rats (Institute of Medicines, Warsaw): Rough-ground wheat 19%, rough-ground barley 19%, rough-ground oats 19%, bran 20%, fish meal 8%, skimmed powdered milk 3%, fodder yeast 5%, casein 1%, vitamin mixture 1%.

The animals used for the experiment were operated on after they had been anaesthetised with ether. The method of operating was described in the study by Dzierżykray-Rogalska (1963).

The rats were dissected under ether anaesthesia and the pancreas removed for fixing in a Bouin's fluid, Lison-Vokaer's fluid and sublimate. The material was saturated in paraffin, then cut into specimens 6 μ thick and stained by the following methods:

a) haematoxylin and eosine,
b) Azan and Mallory method,
c) fuchsine aldehyde — Gomori method (1950),
d) chromic haematoxylin and phloxin — Gomori method (1939).

At first it proved extremely difficult to distinguish the different kinds of cells and it was not until use was made of the technique described in the studies by Gomori, 1939; Hellestrőme & Helman, 1960; Bangle, 1956; Scatt, 1962; Lovene & Feng, 1962; that satisfactory results were obtained. The best results were obtained from use of the method with chromic haematoxylin and phloxin — Gomori, 1939.

Lacy (1957) in examining the cells of the islets in the guinea-pig, dog, rabbit and rat emphasised the special difficulties encountered in diagnosing the cell types of the rat pancreas. In the other animals the cells of the islets can be easily distinguished by their shape and distinctive staining of the cytoplasm granulations.

In the preparations prepared by Gomori's method (chromic haematoxylin, phloxin), in which no difficulty was encountered in distinguishing A and B cells under a micrometric Carl Zeiss-Jena type glass calculation was made of the percentage of composition of the cells of the islet apparatus. For this purpose calculation was made in several preparations for each individual of the cells of the islets up to 1000, indicating the number of A, B and other cells. In addition the size of the cellular nuclei was measured in both types of cells and readings made of two dimensions in the longest and broadest place of the cellular nucleus.

III. RESULTS OF INVESTIGATIONS

Animals in Group *I* (Control)

The islets of Langerhans in the rats in group *I* are of similar structure, no differences in the structure of the islets being observed between the three sub-groups of animals. The islets of Langerhans in the rats of this group occur in the shape of oval cellular concentrations clearly separated from the exopancreatic parenchyma by the bag of connective tissue. The islets are formed of several kinds of cells. Two types of cells are distinguished in the preparations examined — A and B. The other types of cells, occurring scantily, described by other research workers, could not be distinguished in our preparations.

A cells lie in concentrations on the poles of the islet or in small numbers surrounding the whole islet. The granular elements of the cytoplasm of these cells stain a pink colour with the Gomori method using phloxin, which permits of distinguishing them from B cells. Their dimensions are smaller than those of B cells, which gives the impression when they are

examined that the nuclei of these cells lie far closer to each other than the nuclei of B cells. The average size of nuclei of A cells was 4.7—4,9 μ, and they stain lighter than the nuclei of B cells.

B cells are usually situated in the central parts of the islet. Cells of this type usually form bands between which a large number of sinusoids can be observed. The cytoplasm granular elements of these cells stain a steely-blue colour with chromic haematoxylin the Gomori method. Both the whole B cell and its nucleus have larger dimensions than those of A cell. The mean dimensions of the nuclei of B cells are 4.7—5.1. Within the islets of Langerhans in this group of animals the ratio of A cells to B cells is constant: A cells form 20% and B cells 75% in relation to all the cells forming the islets, 5% being formed by undistinguished cells (Figs. 1 and 2).

Animals in Group *II*

The islets of Langerhans in animals in group *II* (which received 0.1 and 0.2 mg of parotin per 100 g of body weight) do not differ from each other.

The decrease in comparison with the control animals in the number of A cells — they form only small concentrations of several cells each, and can be seen on the periphery of the islet, and also the decrease in the dimensions of the nuclei of these cells (mean 4.3—4.7 μ), are remarkable.

The number of B cells distinctly increases. Neither the dimensions of B cells nor of their cellular nuclei alter in relation to the dimensions of B cells in the control animals.

The ratio of A cells to B cells in this group of animals changes — A cells form 17% and B cells 78% of all the cells of the islets (Fig. 3).

Animals in Group *III*

The islets of Langerhans in animals of group *III* are not of uniform structure: the animals in sub-groups 1 and 2 (dissected respectively 7 and 21 days after the salivary glands had been excised) have islets of Langerhans of similar structure to each other, but differ in comparison with sub-group 3 (animals dissected 35 days after excision of salivary glands).

The islets of Langerhans in sub-groups 1 and 2 exhibit distinctly increased contents of A cells in relation to the islets of the control animals. They form large concentrations, consisting of 25—35 cells arranged round the periphery of the islets. Increase is also observed in the dimensions of the nuclei of A cells (size of nuclei of A cells 4.7—5.3 μ).

B cells in sub-groups 1 and 2 undergo considerable reduction in relation to the control animals. The dimensions of neither B cells nor of their

nuclei alter. The ratio of A cells to B cells in these two sub-groups of animals is subject to considerable change (A cells formed about 25%, B cells about 70%, of all the cells of the islets — Fig. 4).

The islets of Langerhans in the pancreas of sub-group 3 animals (dissected 35 days after excision of salivary glands) are very similar in structure to the islets described in the control animals. The percentage of A and B cells is also similar to that in the control group. Dimensions of the cellular nuclei and cells do not differ from the control dimensions (Fig. 5).

IV. DISCUSSION

The investigations made revealed differences in the structure of the islets of Langerhans in rats subjected to experimental factors.

The islets of Langerhans in animals in group *II* (animals which were given parotin, a specific hormone of the salivary glands) exhibit in relation to the control animals distinct reduction in the number of A cells and decrease in the dimensions of the nuclei of these cells. At the same time the number of B cells within experimental group *II* increases.

Birnkrant & Shapiro (1942), Parhon et al. (1956) found that parotin produces hyperglycemic conditions in the organism. The results of our experiments agree with those of the above authors. It would seem that increasing number of B cells (producing insulin) is the morphological expression of compensation of the balance of carbohydrate metabolism.

At the same time the reduction in the number of A cells (secreting glucagon) which we observed may also prove that the action of parotin is synergic with glucagon.

Animals in group *III* (dissected 7 and 21 days after excision of the salivary glands) exhibit increase in the number of A cells with simultaneous decrease in the number of B cells.

By excision of the parotid salivary glands (which we held to be a hyperglycemic factor, in addition to glucagon) we remove one of the elements influencing the maintenance of a raised blood sugar level. It would seem that this condition is the result of the attempt made by the organism to achieve balance between the action of hypoglycemic and hyperglycemic factors.

Animals dissected 35 days after operation exhibit structure of the islets similar to that of islets in rats in the control group. This condition would seem to be the result of the attempt of the organism to adapt itself to new conditions. It may be that during this period (between the operation and dissection) the functioning of the parotid salivary gland is partly taken over by the submandibular salivary gland (as the result of adaptation).

The condition of return of islets of Langerhans to normal function, and in consequence to structure, have not infrequently formed the object of research in which the authors obtained similar results, i.e. return of the gland to normal, using other experiments (Zajusz, 1956).

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WPŁYW PODAWANIA PAROTYNY I WYCIĘCIA ŚLINIANEK PRZYUSZNYCH NA OBRAZ MORFOLOGICZNY WYSP LANGERHANSA TRZUSTKI SZCZURA BIAŁEGO

Streszczenie

Autorki badały przy użyciu metod histologicznych wpływ czynności dokrewnej ślinianek przyusznych na obraz morfologiczny wysepek Langerhansa szczurów białych.

W tym celu zwierzętom podawano parotynę lub usuwano operacyjnie ślinianki przyuszne. Po podawaniu parotyny stwierdzono powiększenie się liczby komórek B (wytwarzających insulinę), przy jednoczesnym zmniejszeniu się liczby komórek A (wytwarzających glukagon). Uzyskane wyniki pozwoliły na wysunięcie hipotezy, że działanie parotyny (jako swoistego hormonu ślinianek) jest antagonistyczne w stosunku do działania insuliny, a synergistyczne do glukagonu.

Po wycięciu ślinianki przyusznej stwierdzono zmniejszenie się liczby komórek B oraz powiększenie się liczby komórek A. Powyższe wyniki potwierdzają działanie hyperglikemiczne ślinianki przyusznej.

EXPLANATIONS OF PLATES

Plate III.

Fig. 1. Islet of Langerhans in the pancreas of a control rat. A cells lie round the periphery. The central parts of the islet are formed of bands of B cells. Sinusoids can be seen. Magnified 150×.

Fig. 2. Islet of Langerhans of a control rat. A cells are situated in a concentration on the pole (arrow). Magnified 450×.

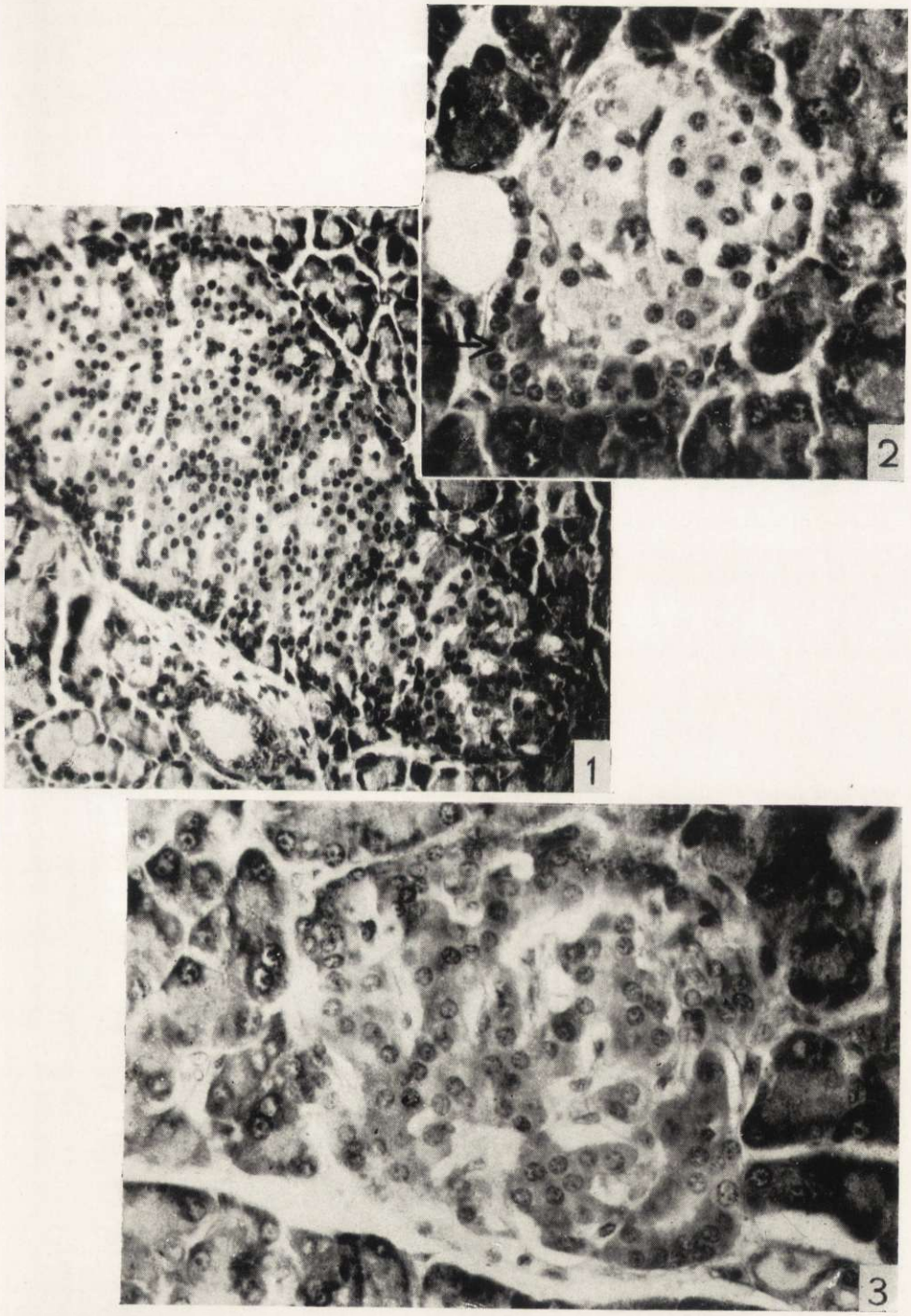
Fig. 3. Islet of Langerhans of animals in group *II*. A cells are arranged in a small concentration on the periphery of the islet. Reduced number of A cells. Magnified 450×.

Plate IV.

Fig. 4. Islet of Langerhans of animals in group *III*, sub-group 1. Large concentrations of A cells (arrow). Magnified 450×.

Fig. 5. Islet of Langerhans of a rat in group *III*, sub-group 3. Structure of islet similar to that in control animals. Magnified 450×.

All sections were stained with chromic haematoxylin and phloxin (Gomori, 1939).



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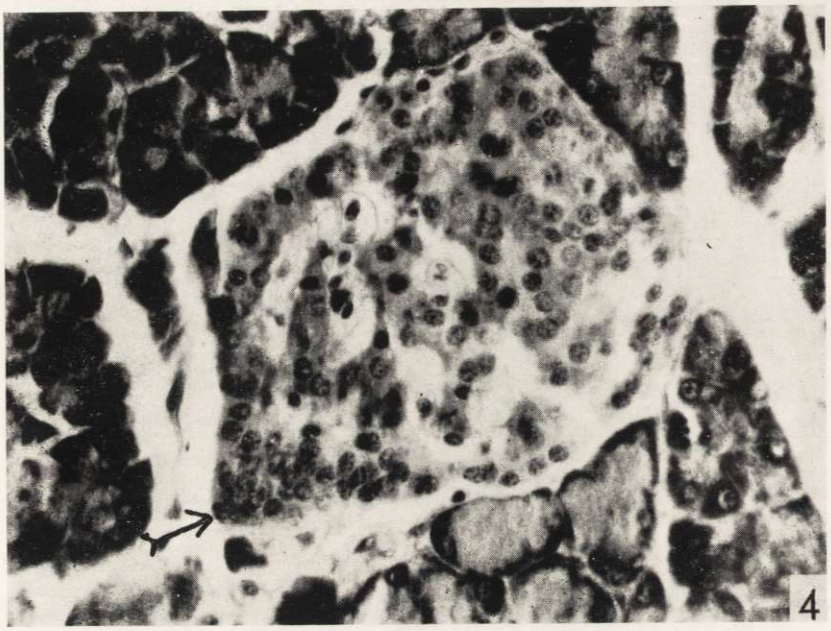

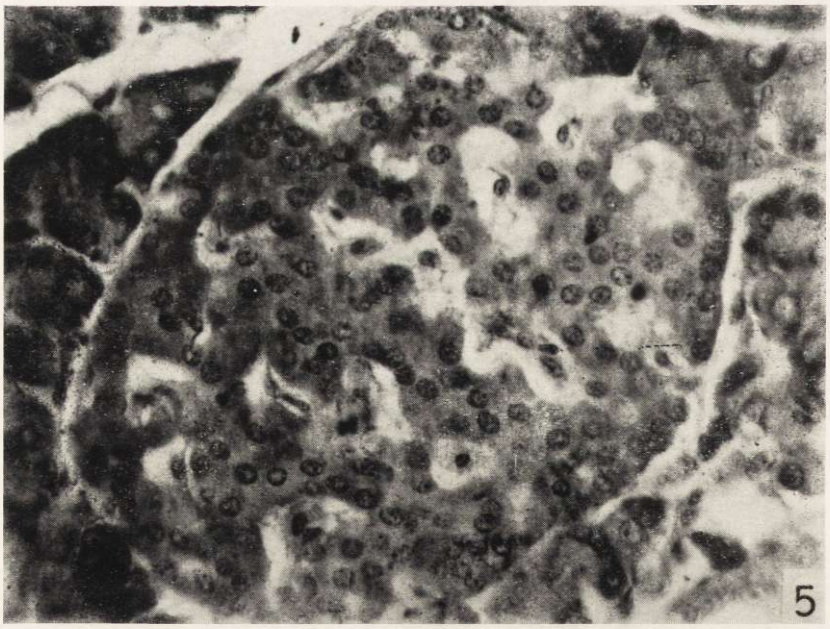

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