PANCREASES AND PAROTIN*

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Since the first recognition that salivary glands may be under endocrine control and/or may actually function as endocrine organs, an overwhelming amount of evidence has been accumulated in support of such concepts. Ogata claimed that bovine parotid glands elaborated a hormonal substance and in 1935 he proposed a "Salivary Gland Endocrine Theory" (Ogata, 1935). He has continued to present results of such studies along with additional evidence to support his original hypothesis (Ogata, 1944a. b). Ito (1960) and associates isolated, from bovine parotid glands, an active principle which they called "Parotin". This substance, a homogenous protein, has the ability to induce specific biologic activities (Ito *et al.*, 1952; 1954). Continuing studies have shown a similar substance to be present in the salivary glands of other animals, as well as in human saliva (Ito and Okabe, 1955) and human urine (Ito *et al.*, 1954).

It has been found that certain morphologic changes take place in particular tissues and organs following the periodic administration of salivary gland extracts (Greentield, 1956; Fleming, 1959; 1960). It was also found that brain transplants of the salivary glands had a higher survival rate when parotin was administered (Fleming, 1961). It was found that certain changes took place in salivary glands and pancreases *in situ* of mice when parotin was administered (Fleming, 1960).

Experimentally induced alterations in the pancreas are important because of their relation to glycosuria and serum glucose levels. These changes result from modifications in the granules in the cytoplasm of β cells. These granules are presumed to be the precursors of insulin (Lukens, 1959). It is well-known that certain hormones and/or hormone-like substances have an effect upon the β cells of the islets of the pancreas (Kinash and Haist, 1955; Lukens, 1959; Ogilvie and MacLean, 1960; Lever *et al.*, 1961).

Since it has been postulated, as mentioned above, that the salivary glands and/or their extracts may actually function as endocrine organs, or may be under endocrine control, it is important to determine the effect that parotid gland extracts may have on the pancreas. Aspects of this problem were investigated in two different manners: (A) to determine what effect there might be on the intact pancreas following periodic parotin injections; (B) to determine what effect there would be on pancreatic tissue transfers when the host animals were given periodic parotin injections.

This communication will attempt to answer both of these questions.

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METHODS

(A) The effect of parotin on the *in situ* pancreas of Rockland mice was studied.

Young adult Rockland mice of both sexes, between 60 and 90 days of age received intramuscular injections of parotin every third day until 8 to 10 injections had been administered over periods of 24 to 30 days. Each dose consisted of 0.2 ml of the extract dissolved in normal saline or the equivalent amount of 0.30 mg of parotin for each injected dose. This solution was prepared by dissolving 3.0 mg of the standardized extract into 2 ml of normal saline.

With certain groups of experimental mice an equal number received periodic injections of 0.2 ml of normal saline. These, and other groups of non-injected mice of the same age and weight, served as controls. All animals were maintained separately as to sex. Animals were sacrificed at periodic intervals.

Blood was obtained from the mice by direct heart puncture under nembutal anesthesia. Blood sugar determinations were usually made from individual blood samples collected following periodic injections. For the chart presented with this communication pooled blood from 2 mice was used for each period and the mean average of the readings is represented on the chart for each point. Gibson's modification of the Folin-Wu method for glucose was used. (Fig. 1) (Folin and Wu, 1920; D'Amour and Blood, 1948).

Pancreases and adrenals were obtained from animals after bleeding. Buffered formalin was the fixative for sections cut and routinely stained with hematoxylin and eosin. Bouin's fixative was used for sections to be stained with Gomori's Chromium Hematoxylin Phloxine for α and β cells and for the Periodic Acid-Schiff reaction for glycogen. Sections were cut at 7 μ . It was

Fig. 1.

significant that in parotin injected animals where islet tissue appeared more abundant and larger and there also appeared to be proportionately more β cells in the islets, there was a corresponding decrease in the serum glucose reading as compared to the saline injected and non-injected animals. More than 300 mice of both sexes were used for these experiments.

(B) The effect of parotin on anterior chamber homo-transplants of late term guinea pig pancreatic tissue was studied.

Pancreatic tissues were obtained from late term guinea pig fetuses and transplanted to the anterior eye chamber of young adult male and female guinea pig. These transplants were allowed to remain for varying periods up to 45 days before sacrifice and recovery of the tissue explants. A total of 36 males and 28 females were recipients of such transfers. This does not include rejects and discards. Eighteen of the male and 16 of the females received 3 intramuscular parotin injections of 1.5 mg each per week. The remaining 18 males and 12 females served as injected controls.

Upon sacrifice of the gravid females the pancreatic tissues were aseptically removed from the fetuses and washed in cool sterile saline. The tissues were immediately placed in sterile Petri dishes with just enough sterile normal saline to keep them moist. Petri dishes were placed in ice at once and kept there during the transplantation procedures.*

The transplantation procedures to the anterior chambers of experimental animals have been described by this investigator (Fleming, 1956; 1959; 1961). In brief they are as follows: Tissue were cut into 1 mm pieces with a sharp pair of iredeotomy scissors. All adhering blood vessels, connective tissue and debris were removed before loading the tissue into a 16 gauge, specially prepared, spinal trocar. Just prior to this, the recipient host was restrained and several drops of 5% cocaine were placed on the eye to be used, for corneal anesthesia. With a sharp double-edged iredeotomy knife an incision just large enough to receive the tip of the loaded trocar was made superiorly on the corneal side of the limbus only. The tissue was then worked to an inferior position where it was lodged between the iris and the cornea.

RESULTS

(A) Significant morphologic alterations took place in both the exocrine and endocrine pancreas following the administration of parotin. These changes were accompanied by a depression in the serum glucose levels in both males and females, which was sometimes quite marked. This depression serum glucose was usually evident from the 2nd through the 8th or 9th injection.

The representative changes taking place in the exocrine pancreas may be noted by comparing Figures 2 and 3. Acinar groups from a saline injected animal are represented in Figure 2 and from a parotin injected animal in Figure 3. The serum glucose readings were 92.1 mg% for the saline injected control and 77.4 mg% for the parotin injected animal. Both animals were males receiving 5 injections over 15 days. Individual cells in the treated pancreas appear larger. This is true for the granules in the cytoplasm which also seem more numerous. As a rule, vascularity was significantly increased and sometimes the vessels became quite large. Such a vessel is shown in Figure 3.

In the parotin injected animals, the islets of Langerhans were enlarged and more numerous with more irregularities along the peri-insular regions and numerous cellular proliferations in and about the ducts were striking. The ducts, blood vessels and the associated islets for a male saline injected control which

* The tissues would soon become very sticky and hard to handle unless kept chilled.

received 3 injections are shown in Figure 4. In Figure 5 these structures are observed for a male parotin injected animal which received 3 injections. The serum glucose reading for the control was 87.6 mg% and for the treated mouse it was 41.2 mg%. It is apparent that islet formations in the treated animal are larger and there are more small islet formations in this instance.

The striking hyperplasia of islets appeared to be associated also with proportionate β cell increases. In Figure 6, a non-injected control section from a male, is shown an islet where the tissue has been stained with Gomori's Chromium Hematoxylin Phloxine to emphasize both the α and β cells. In this figure the nuclei of the α cells are stained dark (red) and the β cell nuclei are light (blue). Figure 7 depicts the same stain in a pancreatic islet where the mouse, a male received 5 parotin injections before sacrifice. The α cells in this instance seemed to be fewer in number and smaller than those observed in Figure 6, the control. In fact, in parotin injected instances all cells usually appeared more uniform in size than those in control sections. The readings for serum glucose were 63.0 mg% for the parotin injected and 89.3 mg% for the control.

It is important to note that neither the islets nor their associated ducts exhibited a significant difference when stained for glycogen by the Periodic Acid-Schiff technique. This is shown for the islet in Figure 8, a control, and Figure 9, where 5 parotin injections were administered. The peri-insular region in Figure 9 is much more uneven than that seen in Figure 8. It should be noted that glycogen was found in similar locations in both instances and extracellular to the β cells.

Figure 10 represents a duct from a male control and Figure 11 represents a duct from a male animal that received 6 parotin injections. The glycogen deposits in the ducts varied for different areas but there were no tangible differences between the parotin injected and the controls.

Where parotin was given, there were alterations in the adrenals. These changes seen in Zone X, or the juxtaposition cells to the medulla where there was an increased cellularity. This resulted in an increase in depth of the zone nearest

Explanation of Figures :

Ac : Acinar cell groups	F : Fat cells
Al : α cells	Gl : Glycogen
Be : β cells	Is : Islet of Langerhans
BV : Blood vessels	M : Medulla
C : Cortex	PL : Plasma cells
Ce : Cells	Tsp : Transplants
Co : Cornea	g : Glomerular layer
Ch : Chains of cells	f : Fasciculata zona
Cy : Cyst	r : Reticulata zona
D : Ducts	x : Zone x juxtaposition
Dc : Descement's membrane	

* Figures with one star were stained with Gomori's Chromium Hematoxylin Phloxine.

** Figures with two stars were stained with the Periodic Acid-Schiff technique.

All other figures were stained with Hematoxylin and Eosin.

Fig. 2. Exocrine pancreas $\times 720$

Fig. 3. Exocrine pancreas $\times 720$

Fig. 4. Pancreas to show some islets $\times 288$

Fig. 5. Pancreas to show some islets $\times 288$

Fig. 6. *Islets of Langerhans $\times 720$ Fig. 7. *Islets of Langerhans $\times 720$ Fig. 8. **Islets of Langerhans $\times 450$ Fig. 9. **Islets of Langerhans $\times 450$

Fig. 10. **Pancreatic ducts $\times 240$

Fig. 11. **Pancreatic ducts $\times 240$

Fig. 12. Adrenals $\times 70$

Fig. 13. Adrenals $\times 70$

Fig. 14. Adrenals $\times 225$

Fig. 15. Adrenals $\times 225$

Fig. 16. Fetal pancreas tissue $\times 114$

Fig. 17. Adult pancreas tissue $\times 114$

Fig. 18. Transplant $\times 22.50$

Fig. 19. Transplant $\times 22.50$

Fig. 20. Transplant $\times 720$

Fig. 21. Transplant $\times 720$

Fig. 22. Transplant $\times 57$

Fig. 23. Transplant $\times 57$

Fig. 24. Transplant $\times 720$

Fig. 25. Transplant $\times 720$

Fig. 26. Transplant $\times 720$ Fig. 27. Adult pancreatic tissue in animal that received parotin $\times 114$

the medulla. Changes were also seen in the blood vessels in the cortex and the blood sinuses in the medulla. Furthermore, blood vessels along the periphery of the medulla appeared larger and more prominent.

A control adrenal is shown in Figure 12 and an area prominently featuring the cortex in more detail in Figure 14. These must be compared with Figures 13 and 15 which represent an adrenal from a treated animal which received 8 parotin injections. These are briefly noted here to support the thesis that pancreatic alterations are associated with the adrenals. The control adrenal was from an animal with a mg% serum glucose reading of 110 and the parotin injected animal in this case had a reading of 84 mg%.

In accordance with this increase in size and numbers of islets, and most importantly, what appears to be increases in the numbers of β cells there was a corresponding drop in serum glucose, which was not followed by a degranulation of the β cells. An example of this alteration in serum glucose is depicted in the accompanying chart. The dots represent a series where the animals were anesthetized before sacrifice and the dashes represent a series killed by cervical dislocation.

(B) Anterior eye chamber homotransplants of late term guinea pig fetal pancreatic tissues were studied with reference to the effect of parotin.

This aspect of the study showed that intra-ocular fetal transfers of pancreatic tissues had a more significant biologic activity in parotin injected hosts.

Ductal areas tended to form large cystic areas in the control transplants over

the longer limits of this study. This did not occur when periodic parotin injections had been given. On a day-to-day basis transfers in treated animals showed greater activity and usually grew larger. They showed continually greater variations in vascularity which increased noticeably and became more marked within several hours after each injection.

The degree of development in donor tissue (approximately 60 days) as obtained from late term guinea pig fetuses is shown in Figure 16. Here one can discern young acinar groups and numerous islets of Langerhans as well as duct systems and blood vessels.

Figure 17 shows the pancreatic tissue of a non-treated gravid female, a donor of fetuses in one series. The state of this tissue is quite different from that seen in Figure 27, the latter being pancreatic tissue from a host female that received 17 injections of parotin over a 45 day period.

Figures 18 and 20 show a pancreatic transfer at a low and a more detailed magnification. This eye transfer, which was recovered after 22 days, was made to an untreated adult female guinea pig. Figures 19 and 21 represent a low and a more detailed section of a recovered transfer after the host, a male, received 8 parotin injections over 22 days.

In Figure 19 attempts for the typical islet formation are observed in several areas. One such area is shown at a higher magnification in Figure 21. The duct shown appears to be undergoing a piling up of many cells which was infrequently observed in non-treated transfers after such a period.

Figures 22 and 24 show at low and higher magnification, the state of a pancreatic transfer following 35 days in the anterior chamber. The large cystic areas are typical of the state of these transfers for such a period. The host was an adult male. In Figure 24 some of the numerous small ducts observed just over the cystic spaces are shown in more detail.

Figures 23 and 25 show a low and higher magnification of a recovered anterior chamber transfer after 35 days. This animal, a male, received 12 injections of parotin. Several pseudo-islets may be seen in these figures. One is shown in more detail in Figure 25. It should be noted that some of the cells are loosely arranged in chains. It is important to note also, that where the hosts were given parotin injections there was a more marked response to these transfers. This host reaction to the pancreatic transplants was intensified over longer periods in injected animals, but not in control animals. While lymphocytes were observed more frequently in the treated instances it was also interesting to note numerous plasma cells in the transplants especially near ducts and blood vessels. This was more apparent for longer periods. Plasma cells are shown in Figure 26 in an area of a transplant recovered from the anterior chamber of the eye after 45 days. The host animal had received 17 injections of parotin. By this time many of the non-injected transplants were much smaller.

It is interesting to note that quite frequently the *in situ* pancreatic tissue showed marked changes over the longer periods of the experiment. This is exemplified in Figure 27 and it was also noted grossly that with this reaction the pancreases assumed a fatty appearance in both mice and guinea pigs. A representative section, Figure 27, shows that much of the exocrine pancreas has

disappeared and acinar groups of cells have presumably been replaced by fat cells. In addition, it is likewise significant to note that acinar groups of the exocrine pancreas did not survive for the longer periods in either the treated or untreated cases.

DISCUSSION

This study shows that in addition to effects on other structures, extracts of beef parotid glands can induce morpho-histologic alterations in both the exocrine and endocrine pancreas of mice. It is likewise suggested, in this study, that the depression of serum glucose levels which followed parotin administration was presumably the result of increased β cell activity thus making more insulin available.

The prompt response of the β cells to parotin represents one of the striking phenomena of this investigation. Certain other substances are known to cause changes in the pancreas which could likewise cause alteration in the serum glucose and/or cause a hypoglycemic state. These factors are: giving large amounts of desiccated thyroid to non-hypophysectomized animals, which will increase the pancreatic weight (Kinash and Haist, 1955); the initial of the triphasic responses to alloxan (Scholer and Gaarenstroom, 1958); the continued administration of the growth hormone (Ogilvie and MacLean, 1960); and, the administration of BZ 55 (Fritz *et al.*, 1956; Scholer and Gaarenstroom, 1958).

It is felt that the response of the pancreas in this instance was not at the same level that Metz observed with the hypo- and hyper-glycemic states. Most of the evidence does point, however, to β cell activity as the primary site of the hypoglycemic state (Madison *et al.*, 1959), but it is also important to consider that it is possible for certain other substances such as the sulfonylureas to act directly on the blood sugar without affecting the islets, for their reactions may be independent of and inconsistent with β cytotropic mechanism (Fritz *et al.*, 1956; Borghese, 1959; Madison *et al.*, 1959).

It is likewise interesting to study Hellerstrom's (Hellerstrom *et al.*, 1960) work for he did not corroborate the findings of others in that red blood cells when increased in the islets paralleled an increase in the β cell activity and thus cannot be a reliable indication of insulin synthesis. The present studies suggest that further explanations are necessary for it appeared that the activity of the β cells was at least in keeping with the striking increased vascularity and the number of red blood cells in association with the islets and ducts. The special stain used for α and β cells also indicates that more β cells seemed present in islets of treated animals. Another fact which is important and was brought out by the Periodic Acid-Schiff stain was that the β cells in the parotin injected animals did not show intra-cytoplasmic glycogen which would be evidence of a degranulation of the β cells. This fact is important because the β cells did not break down. Thus, the increased activity of the β cells appeared to be in keeping with the increased vascularity of the islets.

Hellerstrom (Hellerstrom *et al.*, 1960) points out the important fact that the secretory product of the endocrine pancreas may be exposed to the exocrine

pancreas. This could possibly explain reasons for the altered appearance of acinar groups in the pancreases of treated animals.

One of the characteristic results was the alteration in the adrenals. Contrary to the degenerative changes that Ogilvie and MacLean (1960) noted in their study of the effect of the growth hormone on the rabbit pancreas and adrenal, this study found that medullary sinuses were enlarged and that the cortical cells, Zone X, were increased in numbers and depth. The appearance of many of these cortical cells with some in varying stages of mitosis indicated adrenotropic activity, presumably resulting directly or indirectly from the administration of parotin. Luken's in his review (1959) states that in rats the islets are quite resistant to the growth hormone, but are more susceptible to corticotropin action. Thus, if this hypothesis is correct, the ultimate action of parotin may not be directly upon the pancreas, for in this study certain morpho-histologic changes were observed deep within the cortical layer of the adrenals.

With respect to anterior chamber transplants of fetal pancreatic tissues there are several significant points to consider with regard to the gross and histologic behavior of these homografts following the administration of parotin.

Within recent years the literature has been scant on this particular aspect of transplantation, possibly because little success has been achieved in this area either clinically or with experimental animals.

Brooks and Gifford (1959) did not have success with their homografts in dogs neither on a histological basis nor on one of hormonal activity.

Both Browning and Resnik (1951) and Coupland (1960) had better success possibly because they made anterior chamber homografts in mice and rats. Both Brooks and Gifford (1959) and Browning and Resnik (1951) worked with diabetic animals and it is very interesting that the latter investigators achieved success in that islets survived. This difference could possibly be attributed to several factors, the size and state of grafted tissues, the site of transplantation and the species and status of host animals. In considering Coupland's work (1960) it is apparent that the host animal need not be diabetic in order for pancreatic grafts to survive. This is also brought out in the present study.

The important fact to be considered is that the acinar tissue of the exocrine pancreas degenerated and soon disappeared. According to Coupland (1960) this happened in 7 to 14 days following transplantation. Some acinar groups in this study were still evident 22 days after transplantation but the present investigation substantiates in general the previous investigators findings. It could be possible that the exocrine pancreas might be under the influence or domination of the endocrine pancreas and any upset in metabolic activity or in homeostasis that occurs during and after transplantation and before acceptance by the host will mitigate first against the exocrine pancreas.

The next significant fact in the present experiments was the behavior of the ductal systems for they showed greater activity in the parotin injected hosts than in the controls. It is presumed that they were more functional and that the injections of parotin either directly or indirectly stimulated the ducts sufficiently to prevent cystic degenerative alterations.

It is also interesting to note that many of the surviving cells offered a similar

histologic picture with chains of cellular arrangements similar to that seen in some instances where islet tumors of the pancreas were studied (Rogers, 1960; Rosen *et al.*, 1961). The difference with the present studies was the fact that there were often numbers of plasma cells in established transplants located about or near ducts and blood vessels. This might suggest a functional activity of cells within the transplants of the parotin injected animals that was not present in the non-injected controls.

SUMMARY AND CONCLUSIONS

(A) 1. Parotin administration affected both the exocrine and endocrine pancreases of mice.

2. β cells seemed increased and islets were larger and more numerous in the treated mice.

3. Serum glucose was depressed after parotin was given.

4. Ductal epithelium showed proliferation and increased cellularity in the experimental animals.

5. Vascularity was increased in both the exocrine and endocrine pancreases where parotin was given.

6. Adrenals showed increased vascularity and Zone X near the medulla had more cells and was wider in treated animals.

(B) 1. There was better survival of anterior chamber guinea pig homotransplants of fetal pancreatic tissue when parotin was given.

2. Vascular increases to the transplants was observed soon after each parotin injection.

3. Pancreatic islet tissue was more definitive histologically in parotin injected hosts.

4. Cystic formations were more pronounced in the non-treat control host.

5. Ducts appeared to be more functional in the treated hosts.

6. The exocrine *in situ* pancreatic tissue of treated hosts showed a difference in gross appearance and histologically there were unusually high areas of fat cells.

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