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An islet of Langerhans located within the epithelium of a human pancreatic duct*

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Summary. An unusual structure resembling an islet of Langerhans was described in the pancreatic ductal epithelium of a presumably normal human male. Further studies to establish the identities of both islet and duct were performed. The original hematoxylin and eosin section served for histological description, as well as for nuclear volume analysis to characterize levels of beta cell polyploidy. The section was restained with Gomori's Chrome Alum Hematoxylin Phloxine to ascertain the presence of islet-like cells and to quantitate the ratio of beta- to non-beta cells. The section was then restained by the Feulgen technique to confirm proportionality between nuclear volume and DNA content. An apparently normal islet in the same section served as control. The combined observations were consistent with the interpretation that the structure is indeed an islet situated within the ductal epithelium. The almost complete absence of polyploid beta cells, however, and the high ratio of non-beta cells:beta cells suggested that it was a young islet. A similarly high proportion of non-beta cells in the control islet, as well as the frequent occurrence of ducts within, or in close proximity to, other islets, suggested that the entire islet organ was under some form of proliferative stimulus at time of resection. Alterations of the ductal epithelium and the secretory contents suggested that the duct was also in the process of transformation. Although the islet had apparently arisen within the ductal epithelium, the ultimate source of the progenitor cells could not be determined from these studies. The relationship of the ductal islet to other well known forms of islet regeneration is discussed.

Key words: Ductal islet of Langerhans - Beta cell polyploidy - lslet proliferation

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Introduction

Routine microscopic examination of a pancreas surgically resected from a presumably normal 19 year old male revealed the unusual structure seen in Figs. 1 and 2. Its appearance suggested that it was a pancreatic islet of Langerhans growing in the epithelium of a duct but, on further consideration, it was concluded that both the "islet" and "duct" exhibited sufficient variation from normal to warrant further study to establish their identities and biological significance. The section was set aside for later study, at which time the tissue block was irretrievably lost. We felt, however, that even a limited study of this unique structure would be of interest because of its potential to yield useful information on the origin, growth and regeneration of pancreatic islets. We offer our observations in the anticipation that they will elicit the reporting of similar structures by others.

Normal adult pancreatic islets are considered to have a ratio of beta:non-beta cells of approximately 7:3 (Hellerstrom, 1977). There is still some controversy over this (Volk and Wellman, 1977), but most authors report the predominance of beta cells. Islets are also characterized by the age-related polyploidization of beta cells, to the apparent exclusion of non-beta cells (Ehrie and Swartz, 1974; Ehrie and Swartz, 1976; Pohl and Swartz, 1979; Pohl et al., 1981). Approximately 11% of the adult human

beta cell population becomes polyploid, a figure extrapolated from data on whole islet ploidy (Pohl and Swartz, 1979). In all studies from this laboratory, beta cell nuclear volume has been shown to be proportional to DNA content, which permits an estimate of degree of ploidy by nuclear volume determination. In diabetic humans (Pohl et al., 1981), as well as in mice with inherited forms of diabetes (Ehrie and Swartz, 1976; Pohl and Swartz, 1979), the proportion of polyploid beta cells is significantly elevated.

The ratio of beta:non-beta cells and percent polyploidization of beta cells were selected as relatively simple and reliable indices with which to compare the "ductal islet" with apparently normal islets in the same section. The morphology of the "duct" in which the "islet" was found was compared to apparently normal ducts in the same section.

Materials and methods

Case history

The patient was a 19 year old male who suffered two gunshot wounds to the left upper quadrant of the abdomen and was admitted in shock to the emergency room of Louisville General Hospital with a systolic blood pressure of 60 mm Hg. He was transferred immediately to the operating room where, after being stabilized, an exploratory laparotomy was performed. Two perforations of the stomach and colon were found, together with severe lacerations of the left kidney and the tail of the pancreas. The patient underwent colostomy, left nephrectomy and partial pancreatectomy. Seven days later the patient had recovered sufficiently to be transferred to another hospital, where he was discharged two days later in good health.

The pancreatectomy specimen measured 3 x 2 x 1 cm and showed hemorrhages on the surface. Interlobular hemorrhage was noted by light microscopy. During the patient's stay in Louisville General Hospital a blood glucose determination was made each day before breakfast. These ranged from 105 to 145 mg/dl, with an average of 122.

The patient was contacted 8 years after the injury. He claimed to be in good health and has not experienced any sequelae to his gunshot wound.

Technical procedures

Polyploidy. The H & E section containing the "ductal islet" and numerous apparently normal islets was subjected to nuclear volume analysis for determination of percent ploidy (Ehrie and Swartz, 1976). Since only beta cells become polyploid, determination of total islet polyploidy accurately reflects the percentage of polyploid beta cells (White et al., 1985). In the "ductal islet", all of the nuclei whose equator appeared in the section ($n = 240$) were measured with a Ziess Particle Size Analyzer (White and Swartz, 1980). An apparently normal islet of comparable size was selected as a control and all of the nuclei which met the criteria used for the "ductal islet" were measured in the same manner ($n = 241$). All nuclei in each islet were examined with a $\times 100$ oil immersion objective to determine their suitability for measurement, and each nucleus selected was marked for measurement on the enlarged photograph employed for nuclear size analysis.

Because the control islet contained too few measurable nuclei to resolve the polyploid nuclei present as a distinct class (at least 1000 nuclei are usually required for good resolution by this method), the same nuclei were remeasured more precisely by tracing them with a camera lucida and determining the areas of the tracings by planimetry. From the areas and the magnification, nuclear volumes were determined and compared to the data yielded by the Particle Size Analyzer. This procedure was not necessary for the "ductal islet" since polyploid classes were not present.

To establish the diploid nuclear size distribution for reference, 150 nuclei of exocrine cells, known to be diploid in the human (Ehrie and Swartz, 1974), were measured by particle size analysis.

Ratio of beta to non-beta cells. Complete photographic records of the H & E section were made in both color and monochrome, and all measured nuclei were mapped. The sections were then destained (McCormick, 1969) and restained with Chrome Alum Hematoxylin Phloxine (Gomori, 1941). Beta cells were clearly distinguishable from non-beta cells on the restained section. Using oil immersion, islet cells were classified as either beta or non-beta, and marked as such on enlarged photographs of the islets, in order not to duplicate the measurements. Only cells whose cytoplasm could unequivocally be identified with a specific nucleus of known ploidy class were recorded.

Feulgen staining. The slides were once again destained according to the technique described above and restained by the Feulgen method (Stowell, 1945). The staining was positive, but the cells were considered unsuitable for cytophotometric analysis of DNA content because of the lightness of staining. They were, however, sufficiently well-stained for visual confirmation of the relationship between nuclear volume and DNA content.

Mitosis and binuclearity. Mitotic figures and relative occurence of binucleate cells were noted in both "ductal" and control islets.

Results

Histological examination

Apart from its location, the ductal "islet" had a generally typical appearance (Figs. 1, 2). Its pattern of vascularity seemed normal, and its base was continuous with the "ductal" epithelium into which it was incorporated. The main mass of the "islet" protruded into the lumen, and was separated from the surrounding exocrine tissue by a distinct layer of connective tissue continuous

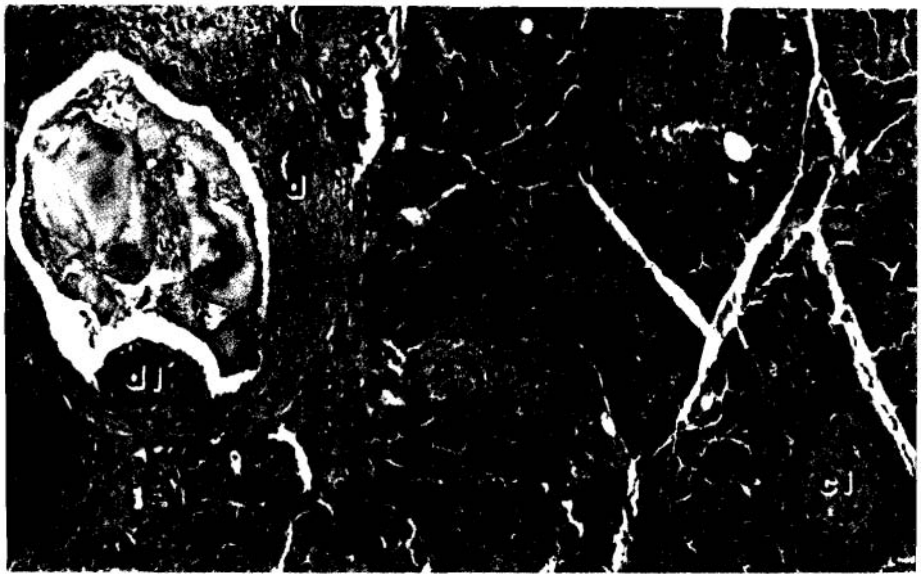

Fig. 1. The "ductal islet" (di) bulging into the lumen of a large duct. One of several small ducts (d) is identified in the surrounding connective tissue. The control islet (ci) is in the lower right-hand corner. × 64

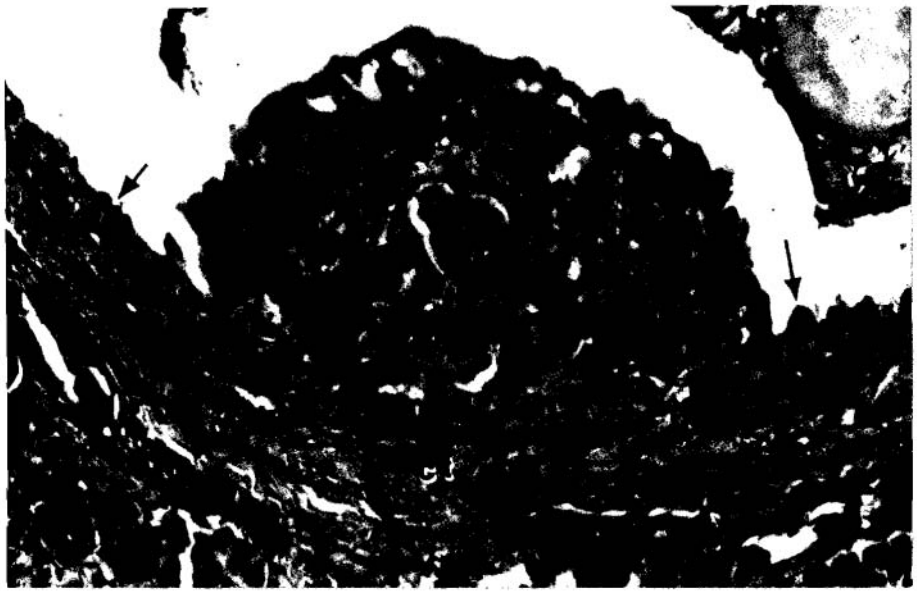

Fig. 2. The "ductal islet", showing continuity with the ductal epithelium (arrows), which resembles transitional epithelium. Connective tissue (ct) forms a complete sheath around the "duct" and "islet". Numerous capillaries can be seen within the "islet". The nuclei of the "islet" vary only slightly in size, reflecting the absence of polyploidy. × 325

with that surrounding the duct. An unusual feature of the "islet" was the absence of large, polyploid nuclei characteristic of normal adult islets. The "duct" in which the islet was situated appeared to be a typical large excretory duct, with certain exceptions: 1) the lining epithelium, usually described as columnar for ducts of this size, was more transitional in appearance (Fig. 2); 2) the basal cells were relatively small and had compact nuclei, while the surface cells and their nuclei were distinctly larger; 3) the free borders of the cells often had convex surfaces that bulged into the lumen, which contained a material that was less acidophilic and more heterogeneous than that seen in nearby ducts. The impression that the modified "duct" was connected to the general duct system was conveyed, in part, by the presence of several cross sections of smaller, more typical, ducts in the surrounding layer of connective tissue (Fig. 1). The adjacent parenchyma, and the remaining islets in the section, were unremarkable (Fig. 1).

Exocrine tissue

Nuclear size analysis revealed only a single class of nuclei (Fig. 3A), which was consistent with past observations. This class, with a mean nuclear volume of 77.96 + 18.86, was assumed to represent the diploid nuclear size class and served as a reference for subsequent measurements. The nuclei of several centroacinar and

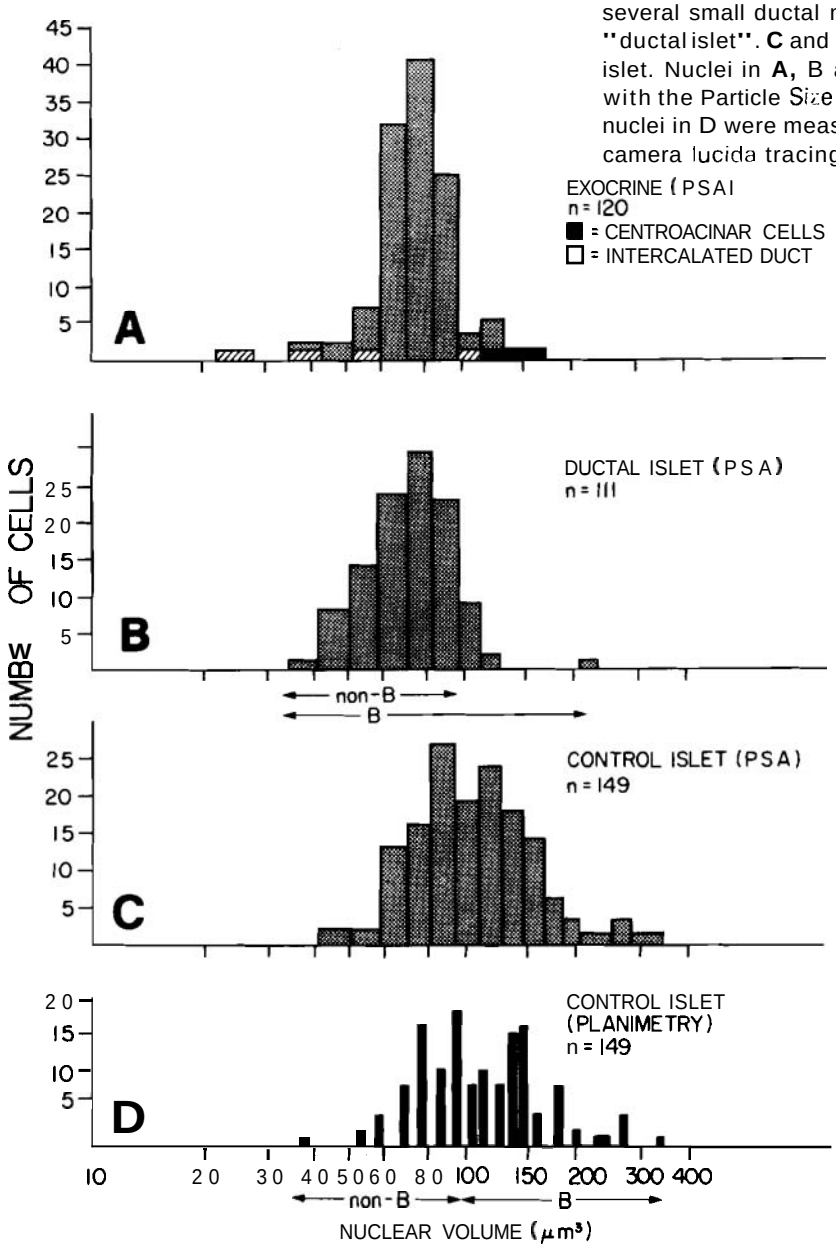

Fig. 3. Histogram of nuclear volume distribution. **A** Pancreatic acinar cells with several small ductal nuclei. B Nuclei of the "ductal islet". **C** and D Nuclei of the control islet. Nuclei in **A,** B and **C** were measured with the Particle Size Analyzer (PSA), while nuclei in D were measured by planimetry on camera lucida tracings.

intercalated duct cells, which were also measured, fell within the range of the diploid acinar cell nuclei.

Ductal** islet **(Fig. 2)

Nuclear size analysis revealed essentially a single nuclear volume class which corresponded closely to that of-the exocrine tissue ($\overline{X} = 76.69 + 22.66$) (Fig. 3B). One nucleus appeared to be tetraploid. When the section was restained by the Gomori technique, 50% of the cells were beta, and 50% non-beta. The sizes of beta and non-beta cell nuclei corresponded over most of the range, but the three largest groups of nuclei belonged exclusively to beta cells (Fig. 3B).

Control islet (Fig. 4)

Nuclear size analysis on the H & E section, using the particle size analyzer, revealed a considerably wider range of values (Fig. 3C), and the nuclei at the lower end of the scale corresponded to those of both exocrine tissue and "ductal islet". Approximately half of the nuclei were larger than the largest nucleus in either the exocrine tissue or the "ductal islet". When the nuclei were remeasured by planimetry, the same volume range was observed, but there was somewhat better resolution of the individual classes (Fig. 3D). Only a suggestion of the peaks expected at approximately 75, 150 and 300 μm^3 was seen,

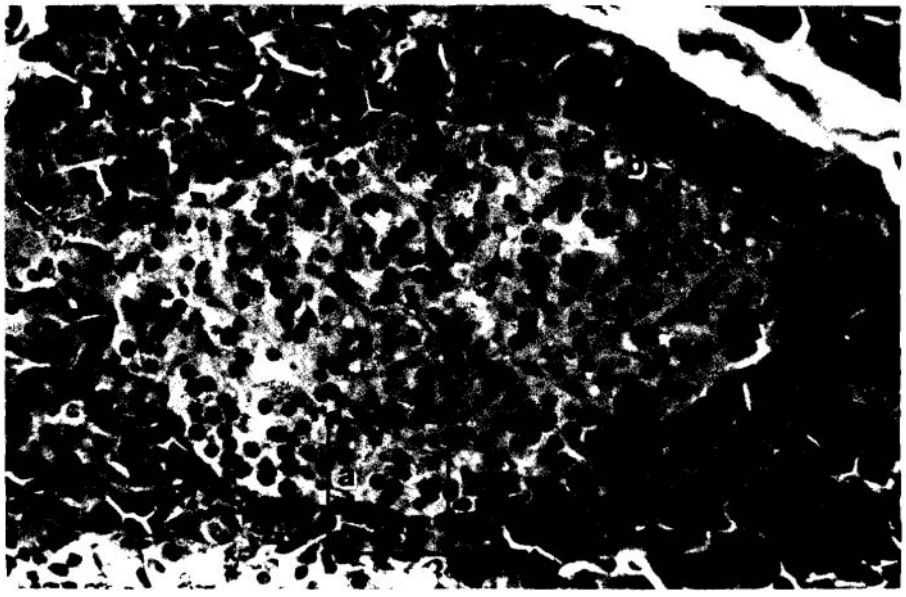

Fig. **4.** Control islet showing large variation in nuclear size, reflecting the presence of different polyploid classes. Binuclear cells are evident. Box a shows the field of Fig. 5 (Feulgen stain), and Box b the field of Fig. 6 (Gomori stain). × 325

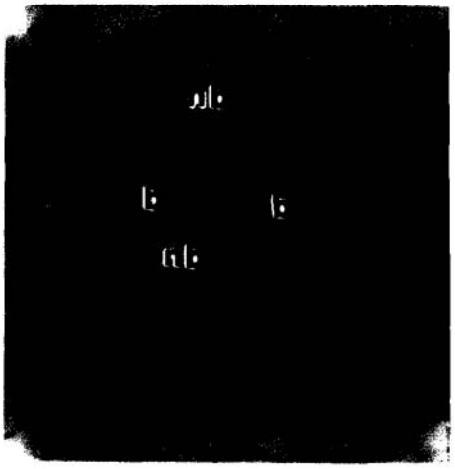

Fig. ***5.*** Feulgen-stained nuclei in Box a of Fig. 4 (control islet). The concentration of dye is comparable in both beta (b) and non-beta (nb) nuclei, providing visual confirmation of DNA-nuclear volume proportionality. x 1270

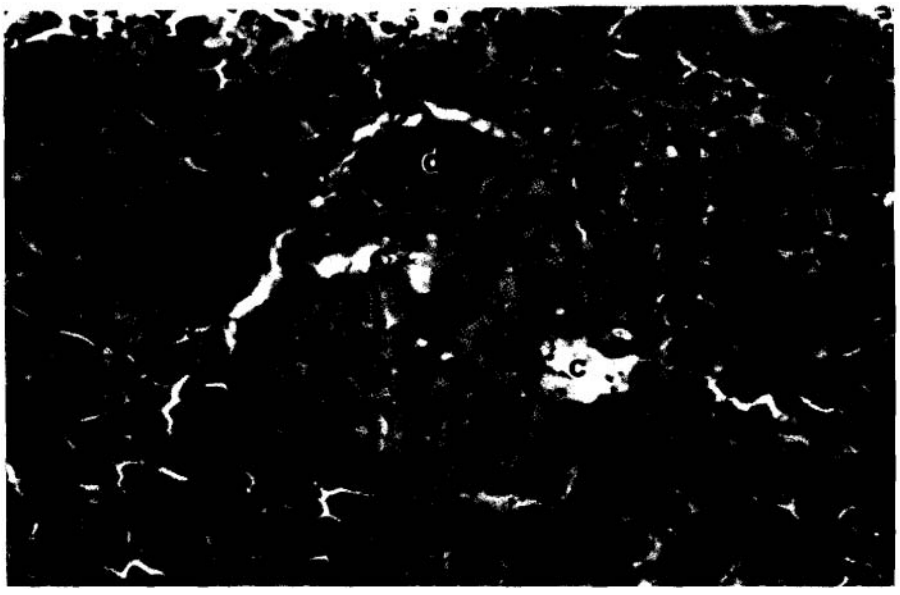

Fig. **6.** Gomori-stained nuclei in Box b of Fig. 4 (control islet). The cytoplasm of non-beta cells (nb), stained red, was photographed with a Wratten # 29 filter (red) and thus appears lighter in the monochrome photograph than the cytoplasm of beta cells (b). Note that the beta cell nuclei (b) are larger than the non-beta cell nuclei (nb), reflecting the fact beta cells of the control islet are all polyploid. x 1700

Fig. **7.** One of several islets in the section showing the close association of ducts (d). Polyploidy and binuclearity are evident. An enlarged capillary (c) is present. x 448

but enough of the largest nuclei were present to confirm the existence of polyploid classes. The Feulgen-stained section (Fig. 5) confirmed visually that the DNA concentration remained constant as the volume varied and, therefore, that nuclear volume reflected ploidy. By conservative estimate, approximately 48% of the nuclei could be considered polyploid.

From the Gomori-restained section (Fig. 6), it was determined that 53% of the nuclei belonged to beta cells and 47% to non-beta cells. Almost all of the non-beta cells corresponded to the diploid class, while almost all of the beta cells were polyploid (Fig. 3D). There was essentially no overlap between classes.

Islet-associated ducts

Many islets showed ducts either within the islet or in very close proximity to it (Fig. 7).

Mitotic figures and binucleate cells

No mitotic figures were found in either "ductal" or control islets. Numerous examples of binucleate cells were seen in both, however (Figs. 2, 4).

Discussion

The evidence presented favors the interpretation that the structure in question is an islet of Langerhans growing within the epithelium of a duct. The general arrangement of the cells, the pattern of vascularity, the presence of the anticipated islet cell types after Chrome Alum Hematoxylin Phloxine staining, the duct-like appearance of the structure in which the "islet" is located, its typical connective tissue investiture, and the presence of characteristic small ducts in the latter, are all compatible with this interpretation. Although both the "duct" and the "islet" exhibit departures from normal, it seems reasonable to view them as modifications of the basic pancreatic structures. It must, however, be stated that the matter cannot be completely resolved from the limited information available here.

The almost complete absence of polyploidy in the islet suggests a young islet, since organs that undergo polyploidization during development are diploid at first and accumulate their polyploid cells gradually with age (liver:Swartz, 1956; pancreas:White, ,1981).

The high ratio of non-beta to beta cells in the "ductal islet" is also compatible with the concept of a recently-formed islet, since non-beta cells appear first during islet development in both man and rodent, followed by beta cells (see Volk and Wellman, 1977). At a particular stage in the early development of islets, therefore, a higher proportion of non-beta cells should be expected. This is in contrast to newly-formed islets in Type I diabetics, in which beta cells comprised the entire islet population at first (Gepts, 1981; Gepts and De Mey, 1978).

Events in adjacent regions' of the pancreas were consistent with the concept that a proliferative response of the islet organ was occurring at time of pancreatic resection. Thus, even in the control islet, almost half of the cells were non-beta, suggesting a recent proliferation of non-beta cells. Secondly, almost all of the beta cells appeared to be polyploid, further suggesting that existing beta cells had been subjected to a polyploidizing stress. A third indication that this particular pancreas was undergoing a proliferative response was the presence of numerous islets with associated ducts. This phenomenon has been observed frequently during proliferative responses of pancreatic islets to various stresses (Logothetopoulos et al., 1970; LeCompte and Gepts, 1977; Like, 1977; Dutrillaux et al., 1978; Nicolesco et al., 1978).

Although the absence of mitotic figures in both ductal and control islets may seem contradictory to the concept of an organ under proliferative stress, the extremely low mitotic rate in adult islets (Hellerstrom, 1977) renders it less likely that cells undergoing division would be detected at any instant by the presence of mitotic figures than if the cell had been exposed to tritiated thymidine. The reason for this is that mitosis lasts only about an hour, while S phase may occupy a third of a day. The presence of numerous binucleate cells, however, is consistent with the idea that the tissue was undergoing polyploidization, since binuclearity is considered an intermediate step in the formation of polyploid cells (Beams and King, 1942; Wilson and Leduc, 1948).

Many of the proliferative phenomena, including polyploidization, described here are associated with diabetes of one form or another, and can be elicited by experimentally-induced hyperglycemia (for review, see White et al., 1985). It has been shown recently that excess dietary glucose can induce accelerated beta cell polyploidization in normal animals (White et al., 1985). It is, however, not possible to attach significance to the somewhat elevated blood glucose levels reported in this study, since they could have been the result of stress induced by the gunshot wound.

It is doubtful that there was sufficient time between the gunshot wound to the pancreas and surgical resection for the observed alterations to have developed. Since a single polyploidization step apparently involves at least two altered mitotic divisions (Beams and King, 1942; Wilson and Leduc, 1948), the time required for DNA synthesis alone would have precluded such dramatic changes as the complete development of the "islet" and the shift of all beta cells in the control islet to the polyploid state. The observations, therefore, favor the interpretation that the reported phenomena existed prior to the gunshot wound.

We have been able to find only one instance of a similar structure having been reported in the literature (Nakamura, 1924). It was discovered in the pancreas of an 11 month old male infant who had died from complications of tuberculosis of the lung (Nakamura, Case 54). The illustiation (Nakamura, Fig. 12) shows an "islet" remarkably similar to the one reported here, except that it occupies most of the lumen of the "duct" with whose epithelium it is continuous. Although it is somewhat difficult to determine from the published illustration, the ductal epithelium does not seem to be as greatly transformed

as that reported here, but the areas contiguous with the "islet" are ,quite similar. From a purely morphological study, Nakamura interpreted the structure, as we have, as a "ductal islet formation". It is interesting that a smaller duct, normal in appearance, can also be seen in the connective tissue investiture of Nakamura's "ductal islet".

With respect to other reported instances of islet regeneration, the ductal islet seems to be unique in at least three ways. First, it was found in an apparently normal human adult, while, without exception, previous reports of islet regeneration have been associated either with instances of human and animal pancreatic pathology, or with pancreatic stress experiments. Secondly, the islet presented here appears to be an integral structure, contiguous with the epithelium of a large duct and bulging prominently into its lumen, whereas previously reported regenerative islets associated with ducts appear either as diffuse or disseminated proliferations of islet cells accompanied by neonatal hypoglycemia or exocrine pancreatic fibrosis (nesidioblastosis) (Bloodworth, 1982; Laidlaw, 1938; Kloppel and Lenzen, 1984), or as accumulations of islet cells in close proximity to, or contiguous with, the outer surfaces of pancreatic ducts (Gepts, 1981). Thirdly, the present islet consists of approximately equal numbers of beta and non-beta cells and is devoid of polyploid cells, whereas in a study of pancreata of type I diabetics (Gepts and De Mey, 1978) "newly-formed" islets are described which are characterized by the presence of small ducts and consist, at first, entirely of beta cells. The presence of obvious nuclear size classes in this type of islet leaves little doubt as to the presence of polyploid nuclei (Gepts, 1981, Fig. 1). Thus, while our data suggest the possibility that the ductal islet is a consequence of a regenerative, or proliferative, process, it appears to be one that differs significantly from those previously described. Its relationship to the other regenerative processes remains to be elucidated.

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