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ABSTRACT

The avian pancreas differs from that of other animals in being composed of two or more lobes and in containing two islet types. Alpha (A) and beta (B) cells are located in separate islets. Islets containing beta cells are called light islets; islets containing alpha cells are called dark islets. The avian pancreas can be divided into exocrine and endocrine portions, and the endocrine portion can be further divided into light and dark islet portions. In the present study involving the duck, the volumes of the exocrine and endocrine portions were measured, and these measurements were used to calculate the relative volumetric distribution of A and B cells. The pancreases of six ducks were used.

The pancreas was fixed in 10% formalin, embedded in celloidin, and serially sectioned (30 μ per section). Areas of both islet types and of the entire section were measured on every tenth slide. Area measurements were then converted into volumes by assuming that each slide was representative of the adjacent ten slides. In addition to the total volume calculations, relative distribution of the islets throughout each lobe was also calculated.

It was found that 99.2% of the duck pancreas is exocrine. Of the 0.8% that is endocrine, 0.3% is light islet and 0.5% is dark islet.

The duck pancreas consists of two lobes with the dorsal lobe divided into three distinct segments. Neither light nor dark islets were distributed uniformly throughout either of the lobes. Both islet types were found along the central axis of the ventral lobe and were arranged in clusters near the ventral surface of the dorsal lobe. The islet clusters of the dorsal lobe tended to be located near the junctions of the lobe segments.

The observations that have been made concerning distribution of the light and dark islets are based on area measurements of serial sections of the entire pancreas, on islet counts made by observation of consecutive serial sections, and on paper models constructed from the serial sections. No attempt was made in the area measurements to differentiate A₁, A₂, and D₁ or clear cells, in the dark islets, although cells appearing to contain few or no granules were observed with the light microscope, especially in the dark islets.

INTRODUCTION

Three or four insular cell types (A, B, and C or D cells) are present in most animal species (Thomas, 1937; Bloom, 1931; Gomori, 1939 a,b, and 1941; Lacy, 1957; Sato, Herman, and Fitzgerald, 1965; Hellman and Hellerstrom, 1960). It has been known since 1922 that B cells produce insulin (Banting and Best, 1922), and it is now well established that at least some of the A cells produce glucagon (Baum et al., 1962; Mikami and Ono, 1962), but the function or significance of the C and D cells remains uncertain.

The avian pancreas is known to contain two distinct islet types (Clara, 1924a; Nagelschmidt, 1939; Oakberg, 1949; Hellman and Hellerstrom, 1960; Mikami and Ono, 1962; Sato, Herman, and Fitzgerald, 1965; Machino, Sakuma, and Onoe, 1966) and to be composed of two to four pancreatic lobes (Clara, 1924b; Nagelschmidt, 1939; Mikami and Ono, 1962). Clara (1924a) and Nagelschmidt (1939) have indicated that the bird pancreas contains proportionately more alpha cells than does the mammalian pancreas.

Mikami and Ono (1962) have reported that the islet-area ratios in the chicken pancreas are 99.24% acinous tissue, 0.41% alpha islet, and 0.35% beta islet; and that no alpha islets appear in the lobes that they call ventral and dorsal. Hellman (1959a) found a B/A cell number ratio of 2.31 ± 0.08 for the adult rat (480 days) and has stated that spherical or ellipsoidal islets are randomly distributed throughout the rat pancreas. It seemed desirable to have endocrine distribution figures...
for the duck pancreas. The subject of this paper is, therefore, the volume, number, and distribution of the islets in the duck pancreas.

MATERIALS AND METHODS

Bensley (1911) described the use of supravital stains (janus green and neutral red) to count the islets of the guinea pig pancreas. We tested these stains on duck, rat, and guinea pig pancreases. They worked for the smaller, more diffuse pancreases of the guinea pig and rat, but did not give useful quantitative results for the larger, thicker duck pancreas. The size of the duck pancreas makes it difficult to count all of the islets before the stain has faded. In addition, Bensley’s method does not distinguish between dark and light islets, an important feature of the bird pancreas. We have found that the following procedures, which yield an easily analysed, relatively permanent set of materials, are more suited to analysis of islet distribution in the duck.

Each animal was exsanguinated and the pancreas removed immediately. The pancreas, duodenal loop, gall bladder, and spleen of the freshly bled duck were removed as a unit from the abdominal cavity and placed in 10% formalin. After an hour, when the material had become sufficiently hardened to permit handling, the pancreas was carefully separated from the rest of the fixed material and placed in fresh 10% formalin solution. It was allowed to remain in the formalin solution for five days and was then transferred to distilled water. Dehydration in alcohol followed, the specimen remaining in each successive solution for five hours, except in absolute alcohol, where it remained for twelve hours. It was then transferred to a 1/1 solution of absolute alcohol and ether, and left for twenty-four hours.

The gland was embedded in celloidin. It was infiltrated with a celloidin alcohol-ether solution for three weeks, remaining one week in 2%, one week in 8%, and one week in 15% celloidin solution (Gray, 1954). Celloidin embedding proceeded slowly, taking about one month. The embedding dish was covered tightly and placed under a bell jar. At intervals of two or three days, the lid was removed for approximately five minutes to facilitate hardening. When the celloidin would retain the imprint of a fingernail, it was immersed in anhydrous chloroform and left overnight to harden. The hardened block was then removed from the embedding dish, trimmed, mounted, and stored in chloroform until needed.

To section, the block was transferred from chloroform directly to 70% alcohol and cut on a sliding microtome at a thickness of 30 μ by the wet method. As each section was cut, it was straightened on the knife and picked up on an alcohol-soaked square of filter paper. These papers were stacked consecutively and stored in a jar of 70% alcohol.

The sections were then removed from the filter paper, clamped onto glass slides with paper clips, and stained, ten at a time, with chromium alum hematoxylin phloxin (CAHP) stain (Gomori, 1939a). Stained sections were dehydrated slowly, differentiated in 70% alcohol, transferred first to a 1/1 solution of 95% alcohol and chloroform, next to a 1/1 solution of 100% alcohol and chloroform, then to a 1/1 solution of xylene and chloroform, and were finally placed in pure xylene. The sections were mounted in picolyte.

For analysis, every tenth section was selected, the final results being based on the assumption that each selected section was representative of the adjacent ten sections (Tejning, 1947). The selected slides were magnified by projection and measurements of the areas of the light islets, the dark islets, and the entire section were made with a transparent millimeter grid. The volumes were then calculated from the areas measured on each section by dividing each of the magnified-area measurements by the square of the magnification of the projection and then multiplying the resulting unmagnified areas by 10 (number of sections represented) times 30 μ (thickness of each section) or by a factor of 0.3 mm (10 x 0.03 mm = 0.3
Total volumes were then calculated by summing these partial volumes. Paper models of each lobe were also constructed and were used to visualize the distribution of the islets within each lobe.

Light and dark islets were counted by scanning consecutive slides. Since the sections were 30 \( \mu \) thick, it was relatively easy to recognize sections through the same islet on consecutive slides. Islets smaller than 30 \( \mu \) in diameter were not counted. The methods of Ogilvie (1937), Oakberg (1949), Mikami and Ono (1962), and of Hellman and his associates (Brolin and Hellman, 1963), which involve the assumption that islets are spherical or ellipsoidal and randomly distributed throughout the pancreas, were not used, because the large light islets of the duck pancreas tend to be rectangular in cross section and the dark islets have a ragged outline and are often elongated or irregular in shape. Because of the irregular shape of the islets and their apparently nonuniform distribution in the duck pancreas, it was felt that a procedure which allowed for accurate measurement of the cross-sectional areas would yield valuable information concerning the actual distribution of the islets. We hope in the future to compare these measurements with ones similar to those developed by Hellman and his associates (Hellman, 1959b; Brolin and Hellman, 1963).

An estimate of the A/B cell ratio (3/2) based on the assumption that A and B cells occupy the same volume was also made. Hellman (1959a) states that, in one experiment, a cell count based on the assumption that A and B cell nuclei were the same size led to an error of 5 to 10%. A similar criticism would apply to the present estimate. In addition, it was further assumed that light islets contain only B cells and dark islets contain only A cells. No attempt was made on the 30 \( \mu \) sections to distinguish conclusively between \( A_1 \), \( A_2 \), and D, or clear cells, in the dark islets and between B and D cells in the light islets. However, cells appearing to have little or no granulation appeared in the dark islets in a ratio of approximately 8 granulated to 1 non-granulated. This gave an A/B/D ratio of 8/6/1, but more work must be done before the significance (if any) of this cell ratio can be stated.

OBSERVATIONS

Distribution of the Islets

It is well known that the pancreas of the adult bird is divided into dorsal and ventral lobes (fig. 1). In the mammalian pancreas (Thyng, 1908), dorsal and ventral endodermal buds appear, but coalesce during development. According to Clara (1924a), the bird pancreas usually contains a third division, the spleen segment. He states that the point of attachment of this small segment to the rest of the pancreas varies from bird to bird. In some birds it is attached to the dorsal lobe only, in some to the ventral lobe only, and in others it is attached to both lobes. In some instances he could find no point of attachment. Clara (1924b) and others (Nagelschmidt, 1939; Machino, Onoe, and Sakuma, 1966; Mikami and Ono, 1962; and Oakberg, 1949) have noted that this segment is especially rich in islet tissue.

We have observed that the duck pancreas is composed of only two lobes, a dorsal and a ventral lobe, and have found that the spleen segment of the duck is an anterior extension of the dorsal lobe (fig. 1). We have also found that the islets in the duck pancreas are not distributed evenly throughout each lobe. We have observed that islets are concentrated in a central cylindrical area in the ventral-lobe, that they are concentrated in clusters near the ventral surface of the dorsal lobe (fig. 4), and that they appear throughout the anterior portion of the spleen segment of the dorsal lobe. The spleen segment of the duck pancreas contains proportionately more islet per unit volume of gland than the rest of the pancreas, as Clara (1924b) and Nagelschmidt (1939) have stated, but we have observed that the large islet clusters which appear in the spleen segment are
almost identical to islet clusters which appear in other portions of the dorsal lobe. The spleen segment of the duck is, therefore, probably best considered as a filamentous extension of the dorsal lobe, in which the characteristic large islet clusters assume a more prominent appearance due to its filamentous character (fig. 1).

In contrast to our observations of the duck pancreas, Oakberg (1949) states that both alpha and beta islets are larger in the spleen segment of the chicken pancreas, and Mikami and Ono (1962) state that the white leghorn cockerel pancreas is composed of four lobes (dorsal, ventral, third, and splenic). Mikami and Ono further state that alpha islets are restricted to those lobes they designate as the third and splenic lobes. (We have observed that clusters of large light and dark islets appear near the junctions of the dorsal lobe segments in the duck.) Mikami and Ono state that the third lobe of the chicken pancreas is a part of the ventral lobe described by previous authors. We have observed only two lobes in the duck. The dorsal lobe, however, is divided into three distinct segments or sublobes (fig. 1), which possibly correspond to the additional lobes observed by Mikami and Ono in the chicken. Clara (1924b) states that the spleen segment is attached variously to the dorsal lobe, the ventral lobe, or to both lobes in different bird species.

**Ventral Lobe.**—The ventral lobe is approximately 4 cm long, 0.5 cm wide, and 0.5 cm thick (fig. 1). Small light islets appear within 1 mm of the periphery of the gland, but all large islets are found in a central cylindrical area 3 mm in diameter, which extends the entire length of the lobe. The islets of the ventral lobe occupy 0.7% of its total volume. Approximately one-third of the islets are located in this lobe.

**Dorsal Lobe.**—The dorsal lobe is approximately 6 cm long, 1.5 cm wide, and 0.5 cm thick (fig. 1). The islets are arranged in clusters near the ventral surface of the lobe, the surface that borders on the intestine. Large islets consistently appear near the junctions of the dorsal sublobes. The largest islets of the dorsal lobe are slightly larger than those of the ventral lobe, and the spleen segment may contain even larger islets. The islets of the main body of the dorsal lobe occupy the same percentage of the total volume as the islets of the ventral lobe (0.7%). The islets of the spleen segment, however, occupy more than 3% of its total volume. The islet cluster contained in this segment is as large as those found in other parts of the dorsal lobe (fig. 4), but, in the filamentous spleen segment, the cluster at places appears to occupy almost the entire volume.

### Islet Structure

**The Large Light Islets.**—Small light islets contain B cells almost exclusively and resemble human islets in staining and in shape (spherical). Large light islets have a regular outline and are rectangular in shape (fig. 2). They are composed of cords or plates of polygonal light-staining B cells (CAHP stain). Many islets are composed of only one cord bent accordion-like on itself, so that the islet appears to contain plates of cells piled one on top of the other, with the plates arranged perpendicularly to the length of the islet (fig. 3). Many large islets contain several rectangular branches and have an overall “Y” shape.

The cytoplasm of the B cells contains a fine granulation, which stains a light reddish color with CAHP stain or a brown-orange color with Mallory azan stain. The cell boundaries are easily distinguishable, and the large oval nuclei contain a light chromatin network. The B cell granules of the light islets never stain as intensely as do the granules of the A cells of the dark islets. No attempt was made in this work to differentiate between B and D cells appearing in the light islets (Machino, Sakuma, and Onoe, 1966; Mikami and Ono, 1962).

Many large capillaries extend across the width of the rectangular large light islets, giving the islets a characteristic striated appearance (figs. 2 and 3).

**The Dark Islets.**—Dark islets (figs. 2, 4, 5, 7) contain both A and D cells and are almost always larger than light islets near them. The average dark islet
occupies four to five times more volume than does the average light islet. In contrast to the regular plate-like structure of the light islets, dark islets have an irregular, ragged outline and no continuous cords. Because of their irregular outline, a dark islet sometimes appears to contain an islet or cord of exocrine cells.

**Figure 1.** A duck pancreas. The mesentery has been removed, and the ventral lobe has been displaced upward. The duodenal loop has been pulled away from the dorsal lobe. Note that the spleen segment is still attached to the spleen and that the dorsal lobe is divided into three distinct sublobes. a) ventral lobe b) dorsal lobe c) spleen segment d) loop of duodenum e) spleen. (Bar equals 5 cm.)

**Figure 2.** Light and dark islets. Note the striated appearance the capillaries give to the light islet, the very regular outline of the large light islet and the contrasting irregular shape of the darker staining dark islet. As usual in the duck pancreas, the dark islets which appear near this light islet are larger than the light islet. a) dark islets b) light islet c) larger blood vessels d) exocrine secretory portion. 30 μm cellloidin sections stained in CAHP. (Bar equals 100 μ.)

**Figure 3.** Higher power of the islet shown in figure 2. Note striated appearance and large capillaries. a) light islet b) exocrine secretory portion c) capillaries. CAHP. (Bar equals 100 μ.)
The A cells contain lightly colored, densely granular cytoplasm. Fine, red-staining granules (CAHP stain) are distributed throughout the cytoplasm. Within each cell, they stain uniformly. However, even in different areas of the same islet, the staining intensity of the granules in different cells varies greatly. The oval-shaped nucleus is polarly located and has a sparse chromatin pattern.

With CAHP stain (Gomori, 1939a), cells appearing to contain little or no granulation were frequently observed in the dark islets and were assumed to be D cells. The A/D ratio appeared to be approximately 8/1, but because no distinction could be made between A₁ and A₂ cells (Hellman and Hellerström, 1960; Brolin and Hellman, 1961) using CAHP stain, and because it was not possible, in the 30 μ sections, to state that cells appearing to be D cells were not lightly granulated A cells, observations were limited to dark islet volume and distribution.

**Light Islet, Dark Islet, and Exocrine Interrelationships.**—Light and dark islets appear in pairs. Most light islets contain few D cells, but at the points where light and dark islets touch, the A, B, and D cells are intermingled. Cells with few or no granules appear to be more numerous at these junctions than in other islet areas.

Almost every light islet is surrounded by several layers of heavily granulated exocrine cells. These “peri-insular caps” (Nagelschmidt, 1939) or “juxtainsular exocrine cells” (Wallgren and Hellman, 1962) are most obvious around the smaller light islets (fig. 4). The more heavily granulated acini of the caps are arranged in a circular pattern, which radiates out from the light islet in a manner similar to the acinar arrangement around a blood vessel or duct. This pattern contrasts sharply with the more random arrangement of the acini of the gland in general. The caps vary in thickness around the larger islets, but always follow the contour of the light islet. Dark islets are often embedded in the peri-insular caps, but appear to have little or no effect on the shape of the caps. Similar caps do not appear around isolated dark islets, although, according to Wallgren and Hellman (1962), the nuclei of exocrine cells near dark islets are somewhat larger than exocrine nuclei far from either light or dark islets.

In some areas of the duck pancreas, large dark islets partially or completely surround one or more light islets to form a characteristic structure we have called circular islets (Nagelschmidt, 1939) (fig. 5). Each circular islet is surrounded by a peri-insular cap composed of heavily granulated exocrine cells. The dark islet portion of the circular islets is composed of alpha cells which take a lighter stain than do the alpha cells of nearby dark islets, and the alpha cells near the islet border are somewhat more intermingled with the bordering acinar cords than in other dark islets. The center of each circular islet is composed of one or two medium-sized light islets surrounded by exocrine tissue.

**Other Structures**

Other structures, resembling islets but composed of tightly packed cells with little cytoplasm and large nuclei, appear in areas around large blood vessels, in solid cords, and in spherical islet-like structures, which are completely encased in a connective-tissue capsule (figs. 6 and 7). Their function is not known.

Materials fixed and stained according to the method used by Lane (1907) to first distinguish A and B cells contain areas of exocrine degranulation. These areas usually appear as lines following the periphery. They do not appear in tissue fixed and stained in other ways, and are probably artifacts of this method. Others (Gomori, 1941; Bargmann, 1939) have also reported difficulties in the use of Lane’s method.

**DISCUSSION AND SUMMARY**

Even a cursory examination of sections of the duck pancreas reveals that there are two islet types: a light-staining group of islets (called the light islets),
FIGURE 4. Dark and light islets in the spleen segment near its junction with the dorsal lobe. Note how close to the edge of the gland the large dark islet is located. a) dark islet b) light islets c) exocrine secretory portion of pancreas d) peri-insular cap. CAHP. (Bar equals 100 μ.)

FIGURE 5. Large dark islet encircling two smaller light islets. a) dark islet b) light islets c) exocrine secretory portion. CAHP. (Bar equals 100 μ.)

FIGURE 6. Dark islets and islet-like structures. a) dark islets b) exocrine secretory portion c) islet-like structures d) blood vessel. CAHP. (Bar equals 100 μ.)

FIGURE 7. Light and dark islets and islet-like structure. a) dark islet b) light islet c) islet-like structure d) peri-insular cap e) exocrine secretory portion. CAHP. (Bar equals 100 μ.)
which contain beta (B) cells and have a regular outline (spherical or rectangular); and a darker staining group of larger islets (called the dark islets), which contain alpha (A) cells and have a ragged outline. The anterior portion of the dorsal lobe, the spleen segment, is especially rich in dark islets.

Analysis of volume measurements derived from serial sections revealed that less than 1% of the duck pancreas is endocrine (0.8%). Of this 0.8%, 0.3% is composed of light islets and 0.5% is composed of dark islets. The A cells occupy a greater volume in the duck pancreas than do the B cells, in spite of the fact that there are more light islets than dark islets. For example, of 25,000 islets in a gland, 20,000 will be light and only 5,000 will be dark. However, because the average dark islet occupies 4.5 times more volume than does the average light islet, and because many light islets are especially small, the dark islets occupy 1.6 times more space than do the light islets.

If it is assumed that individual A and B cells are the same size and occupy the same volume, the A/B cell ratio for the duck is 3/2. If it is further assumed that the nongranulated or only slightly granulated cells (CAHP stain) appearing in the dark islets are D cells, and that A and D cells are the same size, the volume measurements and an A/D cell count in the dark islets indicate an A/B/D cell ratio of 8/6/1. This cell ratio, partially based on islet volume measurement and partially based on a cell count, may vary significantly from a ratio developed from an A/B cell count or from a ratio which incorporates a correction for differences in A, B, and D cell volumes.

Even if only islet volumes are considered, the greater dark islet volume probably indicates that the A cells play a more important role in the endocrine function of the pancreas in birds than in mammals. Because of the isolation of A and B cells into separate islet groups in the duck pancreas and because of the nonuniform distribution of islets throughout the pancreatic lobes, the duck pancreas, like the chicken pancreas, is well suited for measurements of the effect of stresses on the endocrine pancreas and for measurement of the total volumes occupied by A and B cells. Because the location of islets in each lobe is a critical factor in experiments involving partial pancreatectomy (Richards, et al., 1964; Mikami and Ono, 1962), the difference in distribution of the light and dark islets in the duck and chicken pancreases is probably significant. Mikami and Ono (1962) state that the ventral lobe of the white leghorn cockerel contains no alpha islets and that removal of the third and splenic lobes effectively removes the alpha cells and causes hypoglycemia. We have found relatively large dark islets in the ventral lobe of the duck pancreas, so that removal of portions of the dorsal lobe would still leave a significant number of dark islets. However, removal of the ventral lobe and portions of the dorsal lobe near the sublobe junctions would perhaps have an effect similar to that described by Mikami and Ono for the chicken.

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