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From the Histological Department, University of Uppsala, Uppsala, Sweden
(Head: Prof. M. WRETE M. D.)

THE ISLETS OF LANGERHANS IN DUCKS AND CHICKENS WITH SPECIAL REFERENCE TO THE ARGYROPHIL REACTION*

By
BO HELLMAN and CLAES HELLERSTRÖM

With 12 Figures in the Text

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In the pancreas of birds two types of islets of Langerhans can be distinguished, namely the light and the dark. Whereas the B cells numerically completely predominate in the first-named type of islet, the dark islets consist mainly or exclusively of A cells (RUNGE, MÜLLER and FERNER 1956, MÜLLER, RUNGE and FERNER 1956, FERNER 1957, MIALHE 1958). On account of the almost complete topografical segregation of A and B cells the pancreas of birds is a suitable object for studying each of these cell systems separately.

In the present paper a method of silver impregnation modified for islet analyses on thin paraffin sections was applied to chickens and ducks. This procedure has previously enabled us to distinguish two types of A cells in different mammals (HELLERSTRÖM and HELLMAN 1960). Such studies are interesting not only because it has been disputed whether an argyrophil reaction occurs at all in the islet cells of the birds in question (VAN CAMPENHOUT and CORNELIS 1954, DE DUVE 1956) but also because the concentration of A cells in the dark islets greatly facilitates a just estimation of the proportion of cells blackened by silver. The possible argyrophil reaction is correlated with the results of some modern granule stains and histochemical reactions, which in themselves are new contributions to our knowledge of the islets of Langerhans in birds. The existence of two types of islets in the pancreas of birds also raises the question of whether for each of them the distribution of the numbers and volumes of the islets into different size classes is characterized by any definite pattern as previously demonstrated for species with mixed islets (HELLMAN 1959a—d).

Material and Methods

The experiments were carried out on ducks weighing about 2 kg. and on white Leghorn chickens, three weeks old. The animals were killed by decapitation, whereafter the pancreas was immediately excised and fixed in Bouin's, Romeis' or acetate-formalin according to LILLIE (1954).

Paraffin sections of pancreas fixed in one of the above-mentioned solutions were silver impregnated according to the following procedure:

1. After being carefully deparaffinized the sections were refixed in Bouin's solution at 37° C. for two hours.

2. They were washed under the tap for one hour.

3. After being passed through the alcohols up to 95%, the sections were transferred into a solution consisting of 10 g. silver nitrate dissolved in 10 cc. distilled water, 90 cc. 95% alcohol and 0.1 cc. 1 n. nitric acid. Before use the pH of this solution was adjusted by the addition

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of a few drops of concentrated ammonium hydroxide per litre (for a solution diluted 1:6 with distilled water a pH of about 5 was measured by means of a glass electrode). Avoiding exposure to light the sections were left in the solution for 12—18 hours at 37° C.

4. Then followed a quick immersion (< 10 seconds) in 95% alcohol.

5. Development for 60 seconds in a solution consisting of 5 g. pyrogallic acid, 100 cc. 95% alcohol and 5 cc. concentrated formalin.

6. Rinsing in 95% alcohol, 1 min. × 3.

7. At times the sections underwent additional nuclear staining by being passed via the alcohols to distilled water and stained for 15 minutes in Kernechtrot according to § 743 in ROMEIS (1948).

8. Dehydration, xylol and mounting in Canada balsam.

9. In certain cases the study of the silver impregnated cells was followed by examination of the same cells stained according to GOMORI (see below). The introductory preoxidation process in the Gomori method simultaneously served to remove the silver.

In order to differentiate the cell contents of the islets we chose granule stains obtained with GOMORI's chrome-haematoxylin phloxine method and the same authors aldehyde-fuchsin method. The procedure was only applied on the pancreas material fixed in Bouin's or Romeis'. The aldehyde-fuchsin method was combined with a counterstain according to HALMI. The presence of tryptophane was studied by applying the postcoupled benzylidine reaction to 7 μ thick sections of the pieces of chicken pancreas fixed in acetate-formalin. When carrying out the latter histochemical reaction we followed the instructions of LEVINE and GLENNER (1958). By comparing with adjacent sections, impregnated with silver, the tryptophane reaction could be studied with regard to its localization within the A cell islets. For the demonstration of SS and SH groups material fixed in Romeis' was used. This was treated according to BAHR's (1957) modification of the technique described by BARRNETT, MARSHALL and SELIGMAN (1955).

The actual size distribution of the islets was studied on cross sections 4 and 7 μ thick. An equal number of islets was measured from each piece of the body of the organ. The pieces themselves were taken at even intervals and fixed in Bouin's. In each organ so many islets were analysed that the number of islet sections including light and dark islets came to more than 600. In addition a separate analysis of the splenic lobe of the organ was carried out in the case of one chicken. Concerning the light islets their real size frequency distribution was calculated from the apparent one by means of the equation system derived by WICKSELL (1925, 1926). The sectioning scheme, classification of islets and graphic treatment of the material are the same as previously used in similar studies in adult rats and man (HELLMAN 1959 a—d).

Results

Granule stains. The chrome-haematoxylin phloxine method turned out to be especially suitable for identifying islets on sections of pancreas fixed in Bouin's or Romeis'. The islets could easily be distinguished from the surrounding exocrine parenchyma and classified into light and dark ones (Figs 1 a, d). The former type of islet was made up of cell strands consisting of polygonal cells the cytoplasm of which was rich in discreet, light blue-violet granules. In the nuclei, which were mainly ovoid in shape, one or more distinct nucleoli were encountered (Figs 8 a, 1 d). Peripherally in a number of islets we also found a few cells with a pinkish granulation.

Contrary to the light islets within the body region (Fig. 1 d) the dark islet sections often showed considerable divergences from a round or elliptic shape (Fig. 1 a). On the whole the dark islets were less sharply demarcated from the surrounding exocrine parenchyma. Thus small clusters of exocrine cells (Fig. 1 a) were often found in the central part of the dark islet sections. The continuity of these cells with the remaining exocrine pancreas parenchyma could be traced by studies of successive serial sections from the islet in question. Apart from the main constituent of large cells stained bright red, the granule stain used made it possible to

identify in the dark islets along the walls of the capillaries occasional smaller cells with a lighter blue-violet cytoplasm. These cells often bore a certain resem-

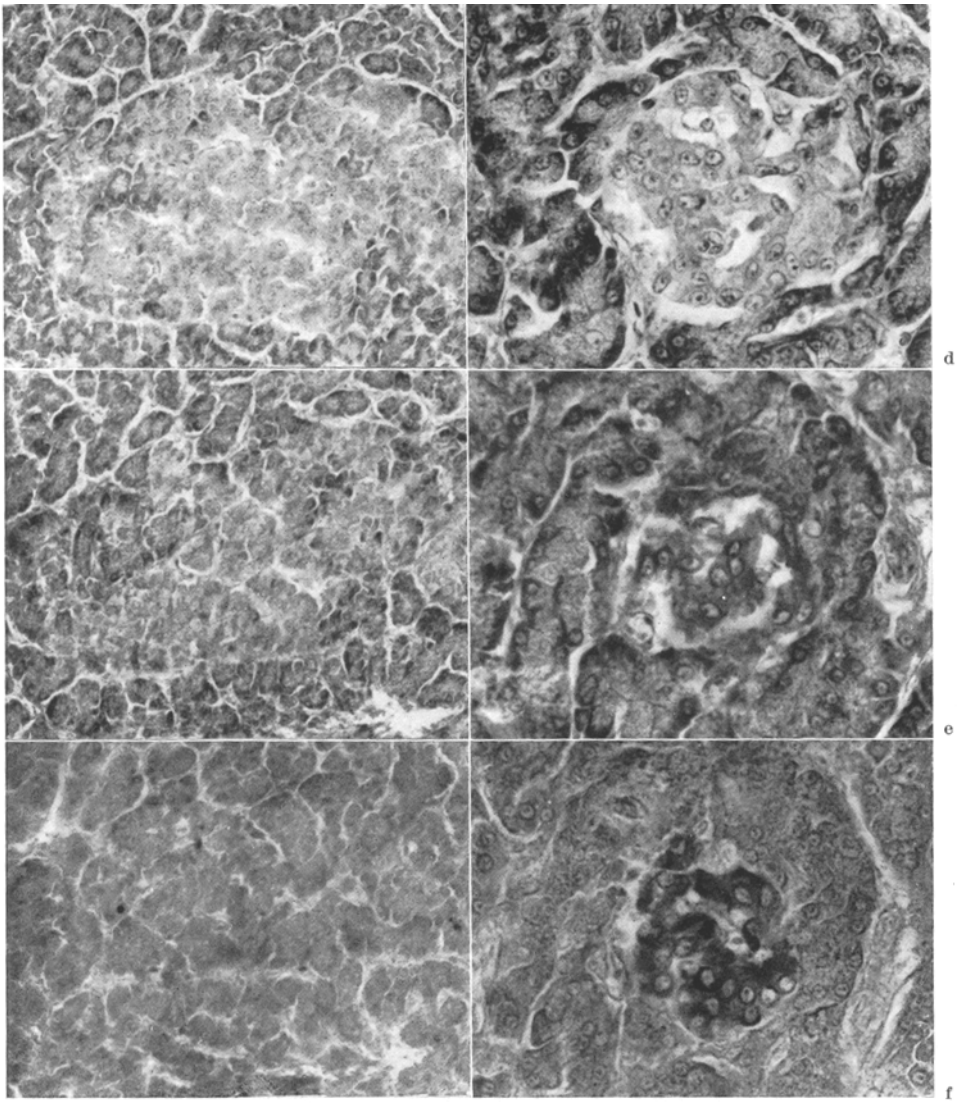

Fig. 1a—f. Result of different stains on duck pancreas fixed in Romeis'. The dark islet in a, b and c is enlarged 160 ×, whereas the light islet in d, e and f is enlarged 400 ×. a Dark islet stained with Gomori's chrome-haematoxylin phloxine. b Same dark islet in an adjacent section where SS and SH groups have been demonstrated according to Bahr's modification of the procedure of Barrnett and coworkers. c Neighbouring serial section of the same dark islet after Gomori's aldehyde-fuchsin with a counterstain according to Halmi. d Light islet stained with Gomori's chrome-haematoxylin phloxine. e Same light islet in an adjacent section where SS and SH groups have been demonstrated according to Bahr's modification of the procedure of Barrnett and coworkers. f Neighbouring serial section of the same light islet after Gomori's aldehyde-fuchsin with a counterstain according to Halmi

blance to B cells, but in most cases they could be regarded as nothing but more degranulated A cell forms (Fig. 2).

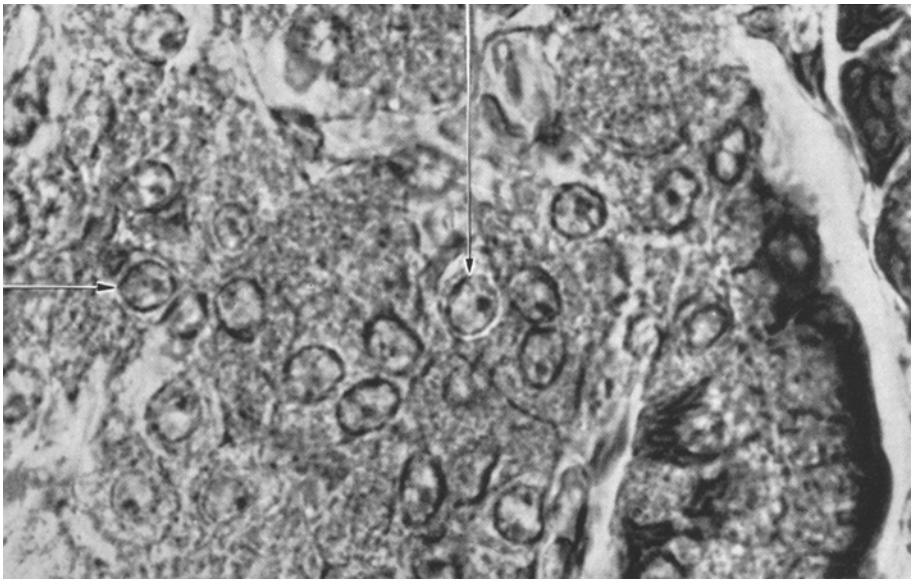

Fig. 2. Dark islet from duck fixed in Romeis' and stained according to GOMORI's chrome-haematoxylin phloxine method. Each arrow indicates a degranulated cell in a characteristic position along the walls of the capillaries. To the right exocrine parenchyma. × 1300

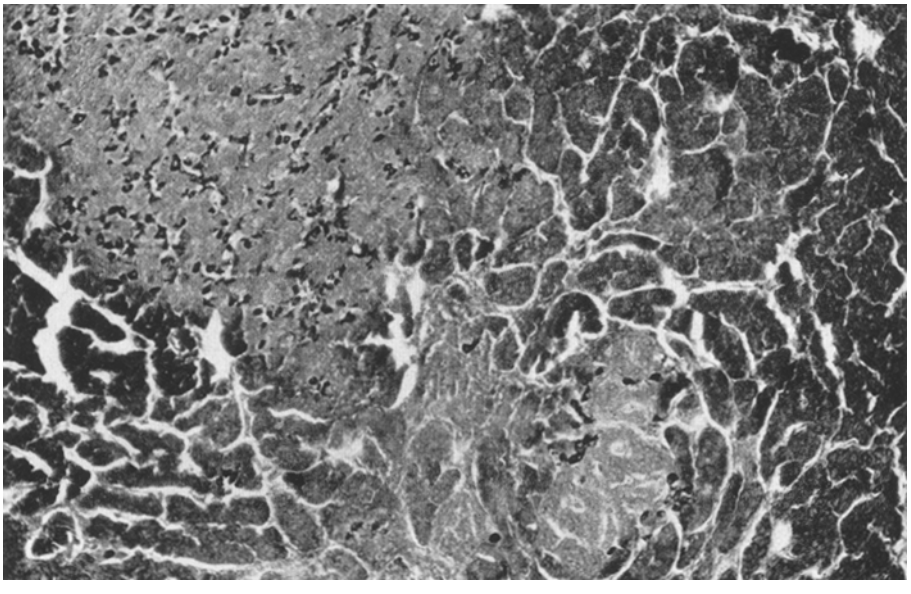

Fig. 3. Survey of light islet (bottom right) and dark islet, both silver impregnated, from duck pancreas fixed in BOUIN's solution. Note the characteristic differences between the two types of islets concerning distribution and number of blackened cells. × 130

After staining with aldehyde-fuchsin characteristic differences between light and dark islets could be noted. Except for a few cells situated at the periphery, the cytoplasm of the cells of the first-named type of islet had a distinctly darkblue

granulation (Fig. 1f). The dark islets, on the other hand, were entirely aldehyde-fuchsin-negative. With the counterstain used it was therefore difficult to distinguish them from the exocrine parenchyma (Fig. 1c).

The argyrophil reaction. The argyrophil reaction was studied with reference to its localization within the light and dark islets. A silver impregnation was obtained with both chickens and ducks irrespective of whether the pancreas had been fixed in Bouin's or Romeis'. Moreover, the silver impregnation procedure was applied to pieces of chicken pancreas fixed in acetate-formalin. Here, too, a blackening

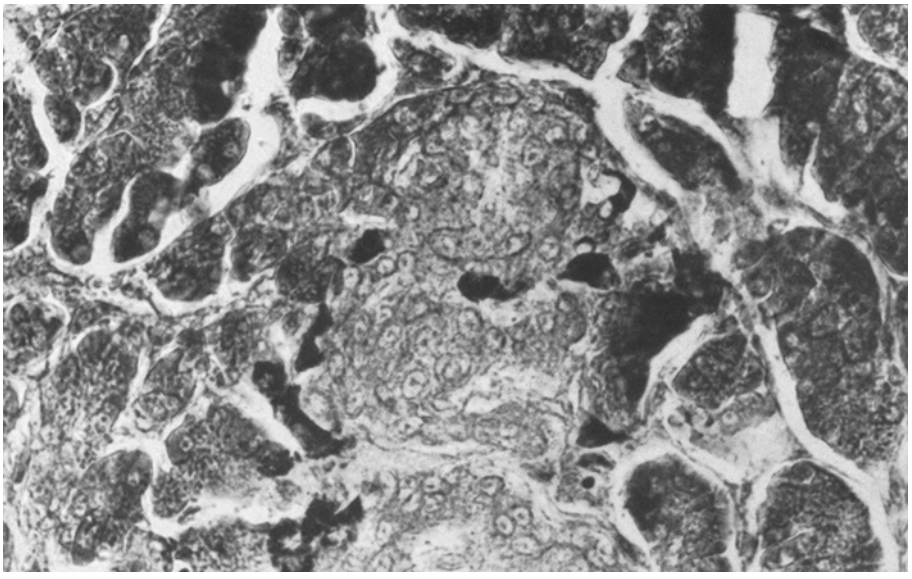

Fig. 4. Detail of the upper part of the light islet in Fig. 3. Compare with Fig. 8 a, where the same islet has been stained with Gomori's chrome-haematoxylin phloxine method after removal of silver impregnation. × 530

of a number of islet cells was obtained, but an evaluation of the results with regard to how the different cell structures had been preserved confirmed the old observation that formalin alone is a poor fixative for tissues to be embedded in paraffin (cf. Baker 1958).

In the light islets silver-positive cells were either entirely absent or rather scarce. The few that were found were usually situated at the periphery of the islets (Figs. 3, 4 and 9a). The frequency of the blackened cells was on the average calculated as less than one percent of the total cell content of the light islets. It should be noted that this value contains a correction for the apparent over-representation of the blackened cells due to the fact that the diameter of these cells is about twice that of the nucleus of the silver-negative cells (Hellman 1959e). A staining with Gomori's chrome-haematoxylin phloxine after the silver impregnation had been removed gave some support to the supposition that the blackened cells are the equivalent of the A cells in the light islets. The results of restaining a light islet after the silver impregnation has been removed are illustrated in Figs. 4 and 8a.

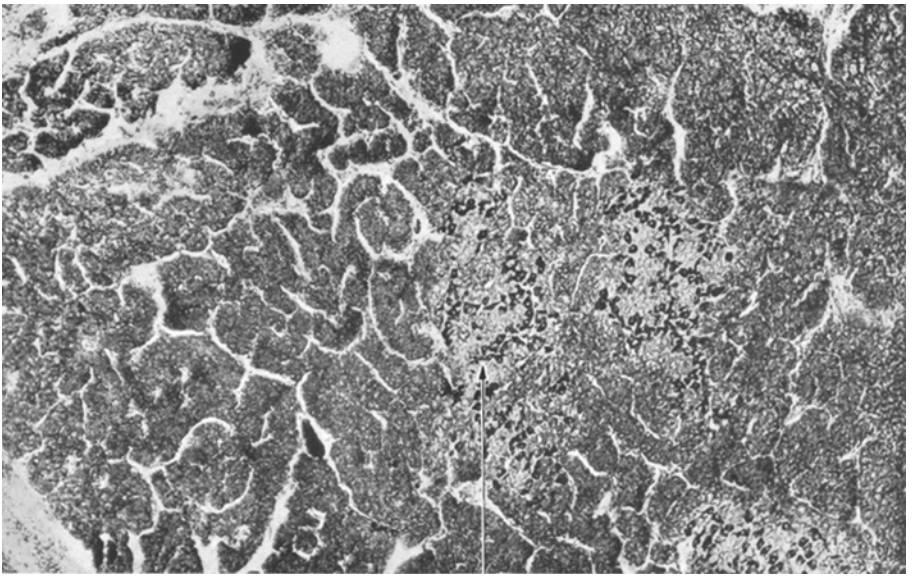

Fig. 5. Survey of dark islet in Bouin-fixed silver impregnated duck pancreas. The arrow indicates the part of the islet shown in detail in Figs. 6 and 7. × 130

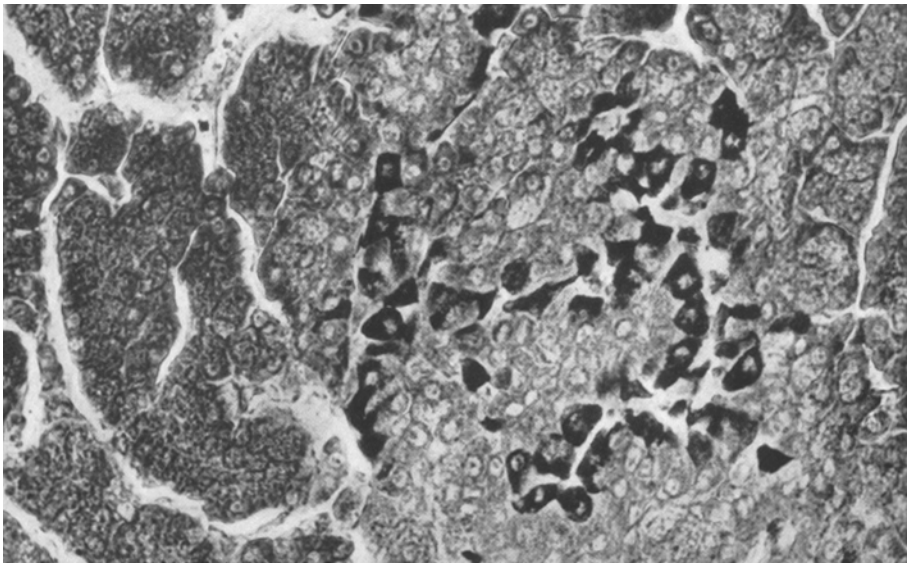

Fig. 6. Moderate enlargement of Fig. 5 showing characteristic localization of silver-positive cells along the walls of the capillaries. × 530

The presence or absence of an argyrophil reaction in the dark islets enabled us to distinguish two different cell fractions in these islets. In the duck the silver-positive cells, calculated to constitute about one fifth of the total cell content, were localized in a characteristic way along the walls of the capillaries

(Figs. 3, 5, 6, and 7). The intimate relations between the argyrophil cells and the capillary system in this animal was further illustrated by the fact that the silver granules in the cytoplasm often had a clearly polar orientation in the direction of the adjacent capillary (Fig. 7). The division into the two cell fractions was distinct, and no obvious transitional forms between the heavily blackened silver-positive cells and the cells that resisted impregnation could be detected. These observations are also valid for the chickens, though to a slighter degree. The argyrophil cells of this bird appeared more slender and were more sparsely granu-

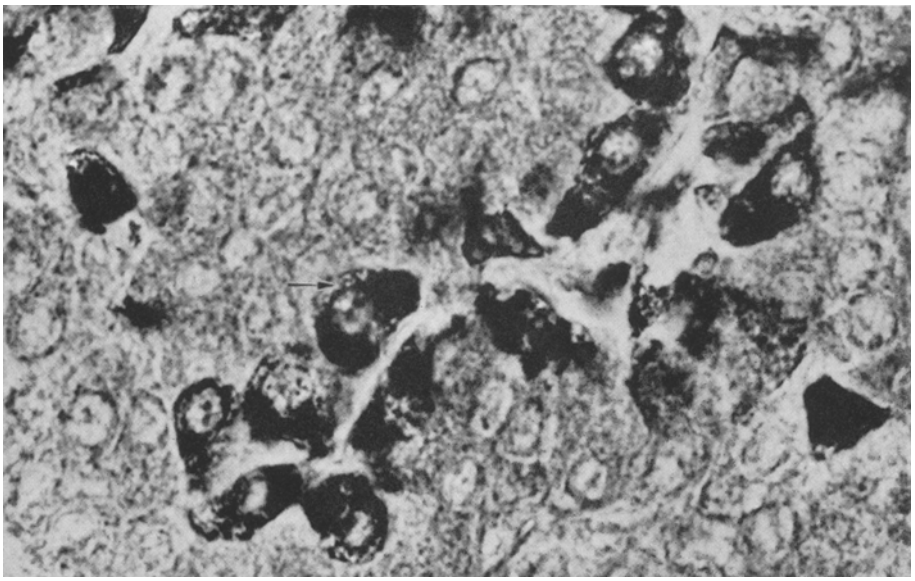

Fig. 7. Enlarged detail of the lower right part of Fig. 6. The black granules are mainly accumulated at the capillary pole of the cells. × 1300

lated (Fig. 9b). An example of a dark islet of a duck restained with chrome-haematoxylin phloxine after a previous silver impregnation had been removed is given in Fig. 8b. Though the variations in the granule content of the A cells, which are clearly visible when only this stain is used, do not appear so distinctly after previous silver impregnation, the silver-positive cells often appear less granulated with this technique as well (Figs. 7 and 8b).

Outside the islets, too, within the acini and the epithelium of the excretory ducts, strongly silver-positive cells could occasionally be observed. Fig. 10 gives an example of the latter localization in a duck.

Histochemistry. The reaction on SS and SH groups was positive within the islets as well as in the exocrine parenchyma (Figs. 1b and e). The two types of islets could be distinguished without difficulty since the dark islets were more weakly stained than the light. After the postcoupled benzylidine reaction for tryptophane the light as well as the dark islets remained practically unstained in contrast to the deep blue zymogen granules of the exocrine cells (Fig. 11).

The size distribution of the islets. Since the shape of the dark islets diverges considerably from spheres or ellipsoids their actual size distribution could not be

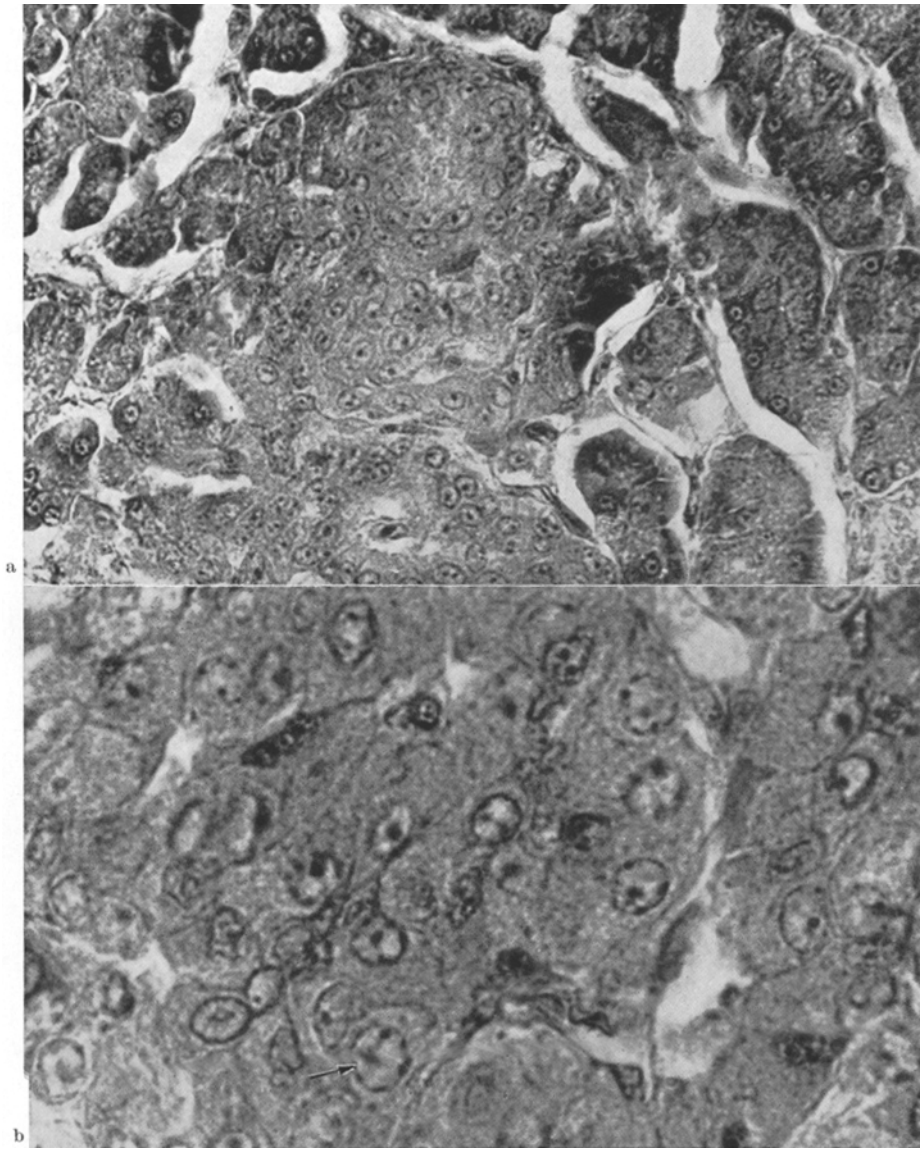

Fig. 8. a Same islet section as in Fig. 4. The picture illustrates the results of an ordinary chrome-haematoxylin phloxine stain after removal of the 'silver stain' × 530. b Same islet section as in Fig. 7 after removal of silver impregnation and subsequently staining with chrome-haematoxylin phloxine. The variation in the granulation of the A cells visible with this stain do not appear so clearly if the section has previously been silver impregnated. However, the cell marked with an arrow in Fig. 7 and 8 b is an example of the fact that cells lending themselves to silver impregnation seem less granulated also with this technique. × 1300

calculated by means of the formulae of WICKSELL (1925, 1926). Studies of successive serial sections revealed, however, that the bigger islets of this type are strongly overrepresented when compared with the size distribution characteristic for the islets of mammals (cf. HELLMAN 1959a—d). The shape of the light

islets and their dispersion in the exocrine parenchyma justified the use of Wicksell's formulae for the calculation of the actual size distribution of these islets at least in the body of the pancreas. Fig. 12 shows the result of such analyses in chickens and ducks. In both cases symmetrical curves were obtained showing that

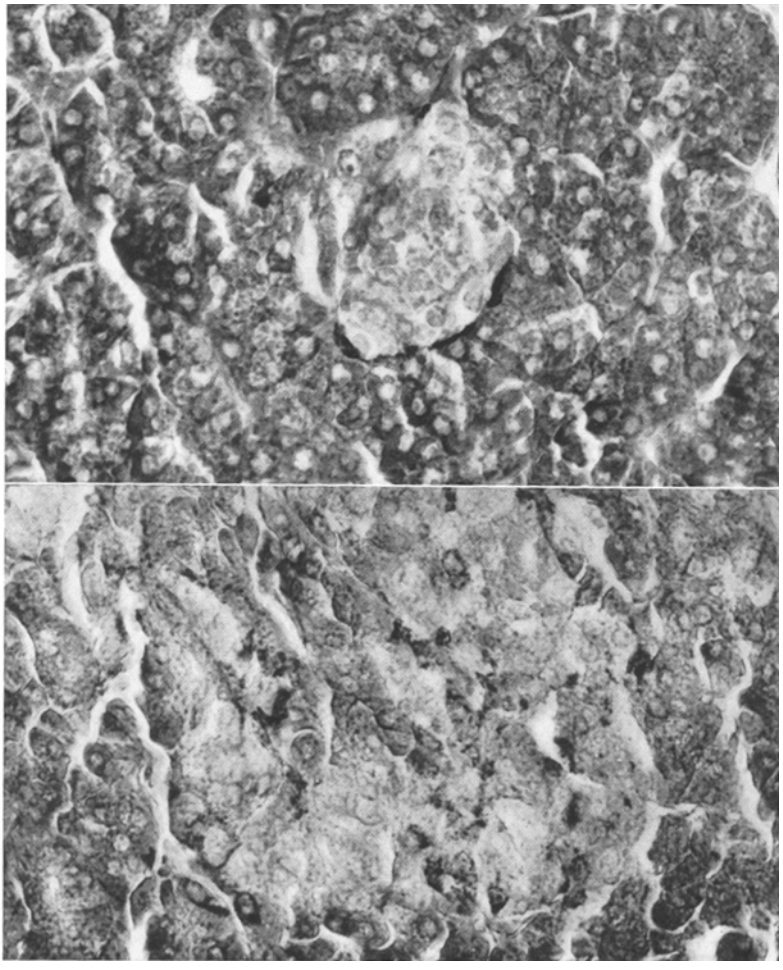

Fig. 9a and b. Bouin-fixed, silver impregnated chicken pancreas enlarged 530 ×. a Light islet. A few elongated silver-positive cells are found in the periphery of the islet. b Dark islet. Numerous elongated slender silver-positive cells are spread over the whole islet. A certain predilection for the capillaries may be noted but is not so pronounced as in the duck

the main part of the islet volume is composed of middlesized islets, while the numerous small islets account for as much of the total volume as the few large ones. Fig. 12 also shows the result of a similar calculation of the actual size distribution within the small splenic lobe of the chicken. Apart from the fact that the curve in this case diverges somewhat from the symmetry demonstrated in the remaining organ, it also appears that the average size of the islets is considerably larger.

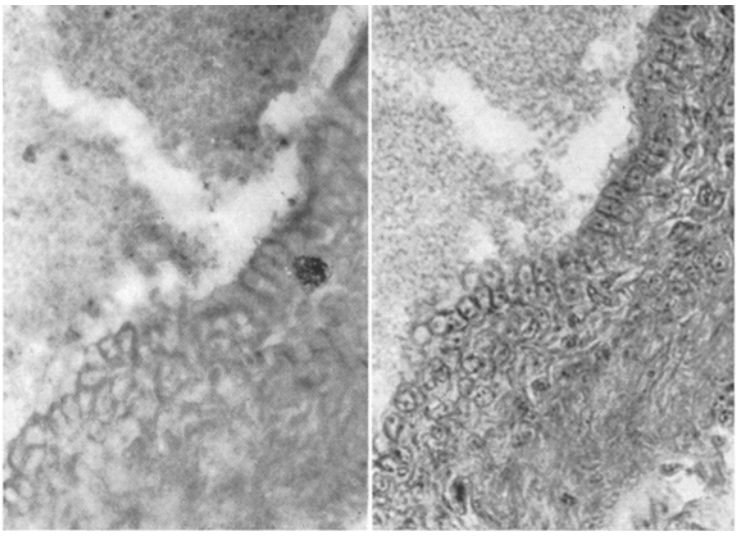

Fig. 10. a Silver-positive cell closely connected with a large excretory duct from Bouin-fixed duck pancreas. × 530. b Same section as in Fig. 10a after removal of 'silver stain' and subsequent stain with chrome-haematoxylin phloxine. The previously silver impregnated cell no longer shows any special morphological characteristics

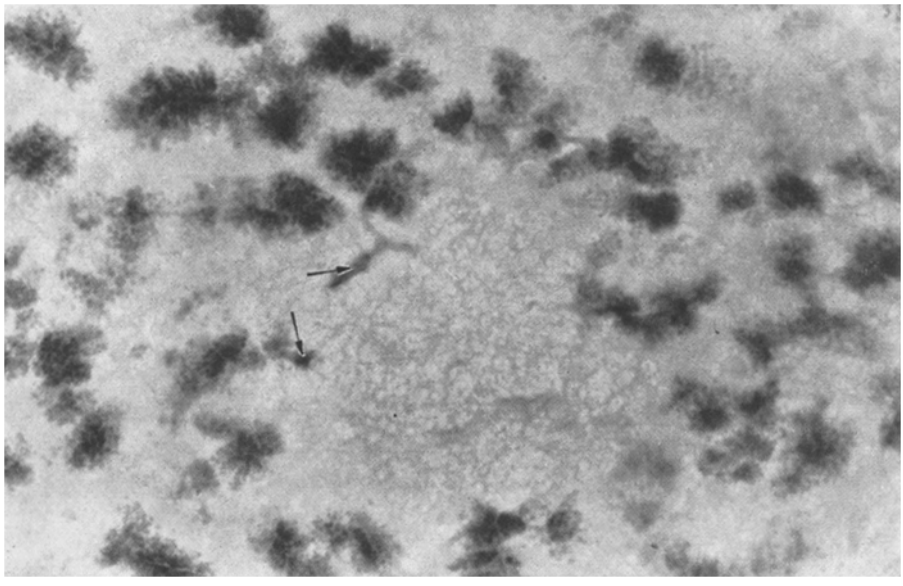

Fig. 11. Dark islet of chicken pancreas fixed in formalin and stained according to the postcoupled benzylidene reaction. The islet, which corresponds to the light part in the middle of the picture, is tryptophane-negative except for the capillaries situated at the arrow. Note that the zymogen granules of the exocrine parenchyma are strongly tryptophane-positive. × 530

Discussion

In the dark islets staining with chrome-haematoxylin phloxine turned the majority of cells bright red, but along the walls of the capillaries cells with a lighter blue cytoplasm could also be observed. In some cases the latter resembled

B cells in the other type of islet. Since the light cells of the dark islets had no positive aldehyde-fuchsin reaction and no significant content of SS and SH groups it is very probable that they do not produce insulin. Instead we interpreted them to mainly consist of degranulated A cells and possibly, but to a slighter extent, of D cells as well. It may be mentioned in this connection that Nagelschmidt (1939) with 'Azan' and Müller, Runge and Ferner (1956) with a modified aldehyde-fuchsin staining technique, have also asked themselves whether a few of the cells in the dark islets are possibly a counterpart of the D cells in mammals. The most powerful argument in favour of the assumption that most of the light

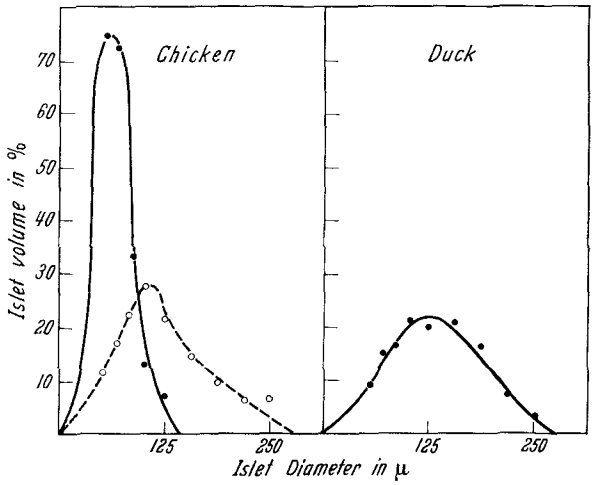

Fig. 12. The percentage distribution of the total volume of the light islets into different size classes in the duck (right) and the chicken (left). On account of the differences in the class width twice the values has been plotted in the first four size classes. The continous lines and solid symbols denote the size distribution within the body of the pancreas. The dotted line and empty symbols show the result of a separate analysis in the small splenic lobe

cells in the dark islets are degranulated A cells lies in the fact that there appeared numerous transitional forms that could not be distinctly differentiated from the majority of A cells richly supplied with red granules.

The question in how far it is possible to silver impregnate islets in different kinds of birds has been discussed by van Campenhout and Cornelis (1953). According to these authors no blackening could be induced in the dark islets of a group of birds including duck, chicken and pigeon, even after maximum silver impregnation. At the same time cells with a distinct argyrophil reaction could be observed in the same sections, but outside the islets. On account of the negative silver reaction van Campenhout and Cornelis argued that it seems incorrect to speak of usual A cells within the dark islets of the birds mentioned. For these cells a new name, e. g. Σ cells should be introduced. The fact that the impregnation technique used by us has actually given a distinct blackening in the dark islets as well sheds new light upon the question. Evidently the above-mentioned authors' result must have been due to their method of impregnation and not to a biological divergence in the A cells of chickens and ducks.

Especially noteworthy is the fact that it is not possible to silver impregnate more than a small proportion of the cells of the dark islets. It may be mentioned

that NAGELSCHMIDT (1939) published illustrations of the same phenomenon in the goose and blackbird. The biological significance of the fact that it is possible on the basis of the argyrophil reaction to divide the cell content of the dark islets into two fractions is as yet unclear. The close relationship of the silverimpregnated cells with the capillaries suggests that they are functionally most active. Thus these argyrophil cells may either be a separate type of cell or only a more active stage of the A cells. The latter assumption is supported by the fact that when the blackening is removed from the silver impregnated sections they appear to correspond to the more degranulated A cells obtained with the Gomori technique. For the other point of view, namely that the silver-positive and negative cells represent two entirely different categories, it may be stated that after adequate impregnation no obvious transitional forms between them could be observed. An identification of an argyrophil fraction among the cells, denoted as A cells when stained with GOMORI'S technique, is possible not only in birds but has its counterpart in all the mammals so far examined by us including man (HELLERSTRÖM and HELLMAN 1960).

In his quantitative studies of the islets of Langerhans in chickens OAKBERG (1949) found that in contrast to the dark islets the light islets were evenly dispersed within the pancreas body. Since these observations appeared to be valid also for the duck and the light islets in that part of the pancreas could be regarded as being spheres or ellipsoids, it was possible to calculate the actual size distribution of these islets by means of WICKSELL'S formulae. The symmetrical forms of the curves thus obtained showed that the size frequency distribution in ducks as well as chickens follows the same mathematical laws earlier described for the rat and man. The fact that the separate analysis of the splenic lobe showed that its volume distribution curve was strongly displaced to the right, i. e. towards bigger islets, agrees with OAKBERG'S statement that the islet sections were significantly larger here than in the remaining organ. On the other hand, it is possible that the asymmetry of the splenic lobe curve does not reflect the true biological conditions, but is merely the result of the more irregular form of islet occurring here (cf. MÜLLER, RUNGE and FERNER 1956). At all events the establishment of the existence of a symmetrical curve within the body of the organ must be of greater significance, for, as OAKBERG (1949) pointed out, in spite of the relative abundance of islet tissue in the splenic lobe the actual number of islets here is so small that the total amount of islet tissue contributed by the splenic lobe is but a small fraction of the total for the whole organ.

Summary

The pancreas of birds is a suitable object for studying the A and B cells separately, since the two cell systems are topografically almost entirely segregated in the form of light (= B cells) and dark (= A cells) islets of Langerhans.

On the whole in the chicken and duck the actual distribution of the light islets into different size classes followed the same regular pattern previously found in the rat and man. In the body of the pancreas, containing the great majority of islets, the volume distribution curves thus appeared symmetrical.

With the silver impregnation method used a distinct argyrophil reaction in both types of islets was obtained on paraffin sections of the pancreas. According to the presence or absence of blackening, the cells of the dark islets could be

divided into two distinct fractions. Especially in the duck the silver-positive cells were grouped in a characteristic way along the walls of the capillaries. Ducks and chickens are not the only animals in which it is possible to identify an argyrophil fraction in what the usual granule stains had shown to be A cells. Parallel studies of various mammals are in complete agreement with these observations. It is, however, still uncertain whether we are here dealing with differences in function, age etc. in one and the same type of cell or with two completely different kinds. No correlation between the argyrophil reaction in the dark islet cells and their content of SH and SS groups or tryptophane could be established.

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Doz. Dr. Bo Hellman,
Histological Department, University of Uppsala, Uppsala Sweden