MORPHOLOGY AND PHYSIOLOGY OF AREAS OF LANGERHANS IN SOME VERTEBRATES.

By LYDIA M. DEWITT, B. S., M. D.,
Instructor in Histology, University of Michigan.

(*From the Histological Laboratory of the University of Michigan.*)

PLATES XII, XIII, XIV, AND XV.

Probably no organ or tissue of the body has been the subject of more thought or investigation than have the areas of Langerhans, especially during the last few years, and yet there are many questions that still remain unanswered. Since a complete review of the extensive literature on this subject would extend this paper beyond the limits that seem desirable, and since relatively exhaustive reviews of the literature have recently been given by Schulze, Ssobolew, Sauerbeck, and many others, it seems unnecessary to do more than call attention briefly to articles which deal with those phases of the subject with which my own work has been especially concerned.

As is well known, the islands were first described in 1869 by Langerhans and later by Laguesse, Opie, Diamare, v. Ebner, Gutmann, and many others. As to their development, Hansemann believes that they are mesenchymal in origin, having no connection at any time with the pancreatic tubules, but arising from the interstitial tissue. Küster and Pearce, investigating human embryos, and Laguesse from the study of sheep embryos arrived at the conclusion that the islands develop from the same anlage as the pancreatic glandular structures. These authors differ, however, somewhat in the details of their conclusions, since Küster states that the islands arise from the ducts, the anlagen, when first differentiated, showing central nuclei, homogeneous protoplasm with a tendency to a band-like arrangement of the cells and having capillaries between the bands of cells.

Pearce, on the other hand, believes that they develop from the pancreatic tubules and are at first solid and later vascularized, a reticulum developing still later. Laguesse concludes that they have originally the same entodermal origin as the glandular acini, but later they are transitory structures, developing from the tubules and changing back into the tubules.

On the general structure of these areas, investigators are practically agreed and the description given by v. Ebner expresses briefly the generally accepted ideas concerning their structure. He states that as a rule, but especially in man, the Langerhans islands are surrounded by a connective tissue sheath, which may be interrupted in places so that island cells and acinal cells are in immediate contact. The arrangement of the cells is peculiar because of their close relation to the large blood vessels, which he regards as venous. These are surrounded on all sides by the cells, like the blood capillaries of the liver lobule, the cells resting directly on the walls of the large blood capillaries without any membrana propria or adventitia capillaris between them. In man, occasionally, the area is divided into smaller compartments by strands of connective tissue and within these compartments again the capillary walls rest directly on the epithelial cells. As a rule, several rows of cells are found between two neighboring capillaries. To some extent in mammalia, but especially in amphibia, single rows of cells are seen between the capillaries, so that each cell is in contact with blood capillaries, on two sides. No excretory duct can be found between the cells. The cells are polygonal in shape, 9–12 μ in diameter (smaller than the gland cells), with very finely granular protoplasm which does not stain well with eosin. The nuclei are ellipsoidal, show a fine chromatin network, and nucleoli which are never so large as those of the glandular cells.

Harris and Gow distinguish three main types of islands in the different species of animals examined : (1) Those having no distinct cells but many small deeply stained nuclei, so that the areas appear not unlike lymphoid tissue. The most characteristic islands of this type are those of the guinea-pig. (2) Those in which the cell outlines are distinct, the cells non-granular and

joined irregularly or forming a network. The areas of the armadillo, potto, and glutton are of this type. (3) Compound cell groups, in which each area is divided into smaller masses by strands of connective tissue. This type is well seen in the human pancreas and also in that of the eagle-owl. V. Ebner, however, states that he finds all these types in the same animal and thinks that the differences depend upon whether the capillaries are collapsed or distended with blood. Diamare (1899) has investigated the areas of Langerhans in teleosts, reptilia, mammalia, aves, and amphibia, finding that in all cases they consist of cords of epithelial cells separated by blood-vessels, but differing in the size and arrangement of the cell cords and in the development of the vascular rete. Pugnat, studying the pancreas of birds of many different species, finds large irregular areas consisting of small elongated cells with indistinct protoplasm and clear nucleus and regards them as lymphoid in nature. In 1904 Rennie published a most important communication concerning the areas of Langerhans in teleostei; in it he reports the results of his study of the areas in twenty-five species of osseous fishes, in all of which very large areas were found in the small islets of pancreatic tissue scattered along the abdominal vessels and among these he finds nearly constantly present a large, so-called "principal islet" a short distance in front of the spleen in the mesenteric fold between the portal vein and the mesenteric artery. Others were found almost constantly in many species. In *Lophius piscatorius*, this principal islet is very large and constant in its position and is independent of pancreatic tissue, being surrounded by a capsule of areolar tissue.

Regarding the connective tissue of the insulæ, Flint says, as a result of his investigations of the human pancreas by the Spalteholz method, that the connective tissue of the islands is definitely and characteristically arranged and sharply contrasted with that of the rest of the lobule. The islands are surrounded by a distinct fibrous capsule in relation with the alveolar framework. Septa or trabeculæ divide the area into smaller compartments containing the cells. His figure shows a septum passing through the center of the area and branches

passing off from it to either side dividing each half into compartments of fairly uniform size. Laguesse, on the other hand, states that there is no connective tissue, but a thin, homogeneous layer, which may be thickened in places ("pseudo capsule"), and that a thin, amorphous sheath accompanies the principal vessel and may spread out over the capillaries. V. Ebner says that as a rule there is no connective tissue or membrana propria between the island cells and the capillaries.

As to the blood supply of the islands, while all agree that they are richly vascularized, there is some disagreement regarding the character of the vessels. V. Ebner regarded them as venous and was supported by Diamare. Kühne and Lea state that the vessels arise partly from capillaries and partly from arterioles. Hansemann finds only capillary connections, and Opie states that "the glomerularnet work is in very free communication with the smallest arteries and that apparently the blood supply is richer than that of other parts of the lobule." Laguesse states that generally a principal vessel enters the island (rarely an arteriole, generally a large branch continuous with an arteriole). In a very recent article, which is profusely illustrated, Pensa has reported the results of vascular injections of the areas of Langerhans in a large number of species of vertebrates. He finds the areas richly vascularized, mostly by a capillary network which is a continuation of the intertubular capillary plexus of the pancreas. In some animals, as birds, guinea-pigs, and dogs, the larger areas may show, in addition to the capillary vessel, a small afferent artery, breaking up into a capillary plexus within the island, the blood being then collected into a single efferent vein. He does not state, however, how he distinguishes the arteries from the veins, and while he states that the connection is usually purely capillary, his Fig. 2 Plate IV, seems to indicate that the connection with larger vessels is very common.

By the Golgi method, Pensa was able also to demonstrate a very rich network of nerve fibers in the areas, the nerves passing along the blood-vessels and also between the cells, and being distinctly different both in number and arrangement from the nerves of the acini.

Dogiel by the Golgi method, and Kühne and Lea and also v. Ebner by injecting the ducts, showed that the areas of Langerhans are not connected by permeable ducts to the excretory ducts of the pancreas, although Lewaschew believed that some of the injection mass penetrated within the island. His results are believed to be due to an extravasation of the injection mass.

Laguesse divides the areas of Langerhans of man with respect to their size into very small, less than 100 μ. in diameter; small, 100μ to 150μ; medium, 150μ to 200μ; large, over 200μ in diameter; and giant forms (very rare), over 400μ in diameter.

After examining ten human subjects, Opie determined that there was in the head of the pancreas an average of 0.366 areas to the square millimeter, 0.36 in the body, and 0.68 in the tail, those in the tail being therefore four times as numerous in the cubic millimeter as in other parts of the pancreas. Sauerbeck in twelve human subjects finds an average of one per square millimeter, while Laguesse in six subjects finds an average of a little less than one per square millimeter and regards anything less than 0.5 per square millimeter as a diminution. He states that the one-hundredth part of the pancreas is area of Langerhans.

As to the distribution of these areas, Opie states that they are four times as numerous in the tail as in any other part of the pancreas and are situated in the center of the lobule, at least in the cat, in which he finds one area in each lobule in the splenic portion of the gland. In man, he says the number is more variable.

My own investigations on the morphology and histology of the areas of Langerhans have been made (1) by reconstruction by the Born wax-plate method of areas of Langerhans from man, cat, rabbit, and rat, and also of injected preparations from cat; (2) by careful study of serial sections of injected and uninjected preparations of pancreas of man, cat, rabbit, rat, guinea-pig, frog, and bird. The tissues were fixed as soon as possible after the death of the animal, in most cases in saturated aqueous solution of bichloride of mercury, and imbedded in paraffin, and serial sections averaging 5μ in thickness were cut. These were stained in hæmatoxylin and eosin, Mallory's connective tissue

stain, Weigert's elastic stain, and in some few cases in Heidenhain's iron-lac hæmatoxylin and in saffranin and *Licht-grün*. Some of the most satisfactory and instructive preparations were those in which there was some congestion so that there was a physiologic vascular injection. These were often more satisfactory for study of the structure than those injected with a gelatin mass, in which the cells of the areas were often more or less changed. For the injected preparations, the simple Berlin blue and carmine gelatin masses were used and also a double injection, the veins being filled with the Berlin blue or carmine gelatin, while the arteries were filled with a granular mass (cinnabar or ultra marine blue.) In this procedure it was difficult to prevent a rupture of the smaller vessels and an extravasation of the granular mass, but some areas of the pancreas often showed a good double injection. In the estimation of the size of the areas, sections near the center of the areas were measured for the first two dimensions, while the third was obtained by counting the number of sections of the series through which the given area extended and multiplying by 5μ, the thickness of the sections. It is easy in nearly all forms to distinguish the central from the peripheral sections, not only by their size but also by the fact that in the center of each area the smallest cords of cells and the largest blood-vessels are found. The volume, as given, is never exact, since it is obtained by simply multiplying the three dimensions, together. However, it answers the purposes of comparision and any more exact method would be exceedingly difficult because of the irregular shapes of the areas.

In the *guinea-pig*, I found an average of 1.07 areas per square millimeter or 1.14 areas per cubic millimeter. As the average volume of these areas was .0091 cu. mm., about one hundredth of the pancreas consists of island tissue. The areas vary greatly in size and shape as may be seen from a comparison of the measurements of the following ten areas:

1.	0.40 x 0.32 x 0.285 mm.	0.036 c. mm.
2.	0.13 x 0.30 x 0.120 mm.	0.0047 c. mm.
3.	0.29 x 0.33 x 0.115 mm.	0.01 c. mm.
4.	0.14 x 0.45 x 0.185 mm.	0.012 c. mm.
5.	0.15 x 0,32 x 0-175 mm.	0,0098 c. mm.

6.	0.13 x 0.18 x 0.11	mm.....	0.0026 c. mm.
7.	0.10 x 0.13 x 0.125	mm.....	0.0016 c. mm.
8.	0.12 x 0.17 x 0.18	mm.....	0.0037 c. mm.
9.	0.15 x 0.2 x 0.25	mm.....	0.0065 c. mm.
10.	0.12 x 0.27 x 0.28	mm.....	0.0098 c. mm.

They are usually oval or elongated in shape with quite regular contour, rarely lobulated or even with marked projections. Near the periphery of the pancreas, the areas are relatively small and spherical or oval, while near the central portion of the gland in the neighborhood of the larger ducts, especially at about the junction of the splenic and middle thirds, the areas are large and prominent. In this region, especially large islands are occasionally found, analogous to the giant areas described by Laguesse in the human pancreas. These often seem to have no connection with the pancreatic acini, being surrounded by the fibrous connective tissue and adipose tissue around the duct. While they vary in shape, many of them are much enlongated, being several times as long as they are wide, some having been found measuring 0.9 to 1.0 millimeter in actual length and 115 to 120 μ in width. In this region also masses of lymphoid tissue are occasionally seen, easily differentiated from the islands by the different character of the cells and by the arrangement of the cells and blood-vessels. The number, position, and relations of these central areas are represented in Fig. 1, a low power drawing of one of the main ducts of the pancreas of a guinea-pig, with the surrounding connective tissue and blood-vessels. In this small mass of tissue, measuring 3.2 mm. in length, five relatively large areas are seen, and three others were just beyond the field of vision. A small amount of pancreatic tissue is seen in the section, but it has no close relation to the areas of Langerhans, which are distinctly encapsulated. Occasionally at the periphery of the pancreas in the adipose tissue of the surrounding mesentery, I have seen areas which appeared to be free. Usually by tracing such areas through the series of sections in which they occur, some few pancreatic tubules are seen in loose or close relation with them.

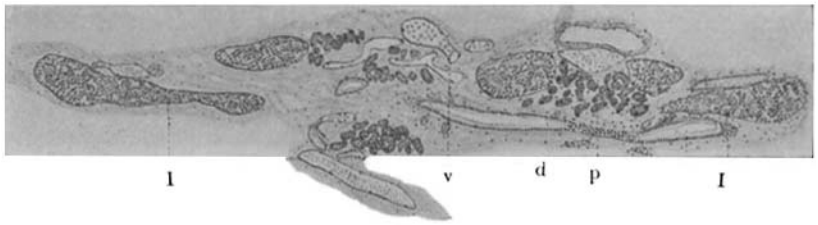


Fig. 1.—Large duct from pancreas of guinea-pig, showing surrounding connective tissue and blood-vessels with a small amount of glandular tissue and five relatively large areas of Langerhans. I, areas of Langerhans; p, pancreatic acini; v, blood-vessels; d, duct. × 25.

The discovery of such relatively isolated islands suggested to me the possibility that isolated areas might be found in the

mesentery of some guinea-pigs, as in the teleostei, as shown by Rennie. Since the observation was made, however, only a small number of guinea-pigs have been at my disposal, and in these I have been unable to find isolated areas at any considerable distance from the pancreas, although, as in the teleostei, strands of island-containing pancreatic tissue accompany the vessels for some distance. With the exception of the relatively isolated areas above described, the insulæ of the guinea-pig's pancreas are in close relation to the pancreatic acini, being separated from them only by the membrana propria, which is often difficult to demonstrate, and sometimes for a distance by a capillary.

The cells are usually described as of two kinds, but to my mind, this classification scarcely covers all the cells found in the islands. (1) The great majority of the cells are, as has often been stated, polyhedral in shape, have homogeneous or nearly homogeneous protoplasm staining faintly with eosin, and have spherical or oval nuclei which are about the size of the nuclei of the acinal cells and show chromatin granules and one or two nucleoli. Many of the cells show distinct cell outlines. (2) There are a few cells with homogeneous protoplasm and very large faintly staining nuclei. (3) Small cells, with small, deeply staining nuclei occur. (4) Large cells with eosinophile granules in the protoplasm and with large, faintly staining nuclei are found. (5) Eosinophile cells with small, excentric, deeply staining nuclei are usually not very numerous and may be found either near the periphery of the island or in the central portion adjoining a blood-vessel. In well-fixed and well-stained sections, I have never found the syncytial appearance described by Hansemann or the masses of nuclei without cell differentiation described by Harris and Gow.

In every respect the structure of the central insulæ surrounding the duct is the same as that of the intralobular areas. The insular cells are arranged in irregular, sinuous bands, sometimes, especially in the central part, consisting of a single row of columnar cells with deeply staining and often quite large nuclei, sometimes of a double row of such cells, while in places, especially at the periphery of the area, the cells are grouped into small masses

The bands of cells follow the arrangement of the blood-vessels, but I failed to find any arrangement of blood-vessels or cords of cells which I could regard as characteristic for the species. In some sections, a larger vessel may be seen entering at one end of the area and passing through the center of the island, dividing it into two more or less equal portions; smaller branches passing off from the sides of this main vessel subdivide each half into larger or smaller compartments, giving a figure not unlike the figure and description given by Flint of the arrangement of the connective tissue in the human areas. Very little or no connective tissue, however, accompanies the vessels. In other sections, the vessel enters at one side soon dividing into numerous larger branches, which pass obliquely through the area in every direction, giving off many secondary branches which frequently anastomose by means of true capillaries.

The capsule, if such it may be called, is very delicate, and the walls of the insular capillaries seem to rest directly on the epithelial cords. When some shrinkage has taken place, however, the capillary occupies the center of a space which separates it from the epithelial cords, and in preparations well stained with Mallory's connective-tissue stain, delicate sheaths of connective tissue are seen to surround the blood-vessels; delicate lines of blue outline the cells, while a very thin capsule of connective tissue separates the area from the pancreatic acini.

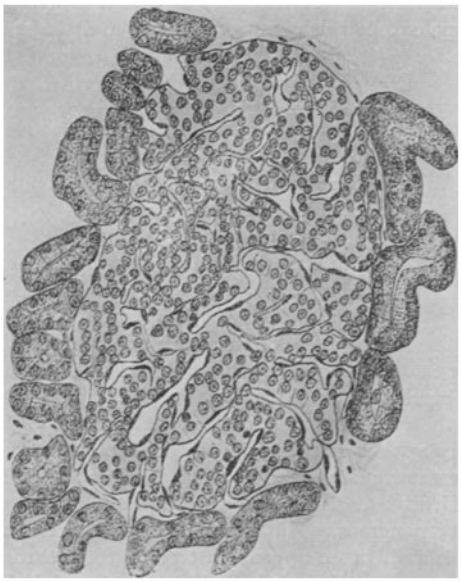


Fig. 2.—Area of Langerhans from pancreas of guinea-pig. Section through center of area. ×20.

The amount of connective tissue in the areas is so much less than

that of the surrounding acini, and the arrangement is so different that the areas are sharply differentiated from the acini by the arrangement of the connective tissue alone. Fig. 2 shows a section through the center of a rather typical intralobular area of Langerhans of the guinea-pig. It is surrounded by pancreatic tubules except at one end, where the area reaches the periphery of the lobule and is limited by the interlobular connective tissue. The large columnar cells, single or double rows of which form the central cords of the area, and the more crowded cell masses at the periphery are well seen. These peripheral masses resemble somewhat the description given by Harris and Gow of the areas of Langerhans of the guinea-pig,—masses of nuclei, not differentiated into cells, and somewhat resembling lymphoid tissue—but the area as a whole is distinctly different.

In the *rat* also, the areas near the center of the pancreas are larger and more numerous than those near the periphery, but I have never, in the material at hand, been able to find the large isolated or nearly isolated central areas so characteristic for the guinea-pig. The measurements given below indicate to some extent the variations in size and shape of the areas of Langerhans in the rat.

1. 0.53 x 0.27 x 0.26 mm. .....0.037 c. mm.
2. 0.50 x 0.43 x 0.32 mm. .....0.0688 c. mm.
3. 0.14 x 0.38 x 0.22 mm. .....0.018 c. mm.
4. 0.22 x 0.23 x 0.40 mm. .....0.02 c. mm.
5. 0.26 x 0.62 x 0.475 mm .....0.076 c. mm.
6. 0.16 x 0.25 x 0.30 mm. .....0.012 c. mm.
7. 0.30 x 0.60 x 0.51 mm. .....0.092 c. mm
8. 0.14 x 0.18 x 0.29 mm. .....0.007 c. mm.
9. 0.17 x 0.51 x 0.465 mm. .....0 04 c. mm.
10. 0.15 x 0.18 x 0.21 mm. .....0.0376 c. mm.

The average size of these ten areas is .0376 c. mm. and, as the average number is 0.7 per mm. in the material at hand, about $\frac{3}{100}$ of the pancreas consists of insular tissue. It is somewhat difficult to be sure of counting areas correctly in the rat, since most of the areas are irregular and lobulated so that often areas may appear as two quite widely separate areas in one section, while in another they unite into one relatively large island. This can easily be seen from Plate XII, Fig. 1, which reproduces a model of a typical area of Langerhans from the rat's pancreas. This island of Langerhans is not nearly so large nor so lobulated as islands often appear, in tracing them through the series of sections in which they occur. It consists of two distinct lobules of insular tissue, well separated at the periphery, but joined at the center into a solid mass. The sponge-like appearance of these areas is well seen in the figure, since, as stated before, the relatively loose, band-like arrangement of the cells at the center is concealed to some extent by the more solid masses of small cells at the periphery, through

which only the small capillaries and the few afferent and efferent vessels pass. In the rat, however, the cells at the periphery are to some extent arranged in irregular cylinders resembling tubules in contour, but having no lumen and having a very free communication with other cylinders. The cells of the rat's insulæ are very similar to those already described for the guinea-pig. Large columnar cells with large nuclei arranged in definite bands consisting of one to two rows of cells occupy the central portion, while at the periphery most of the cells are smaller, the nuclei more crowded and the arrangement less band-like; the blood-vessels are smaller and less prominent. Fig. 3 represents the appearance of a section through the central part of a small area of Langerhans from the rat's pancreas.

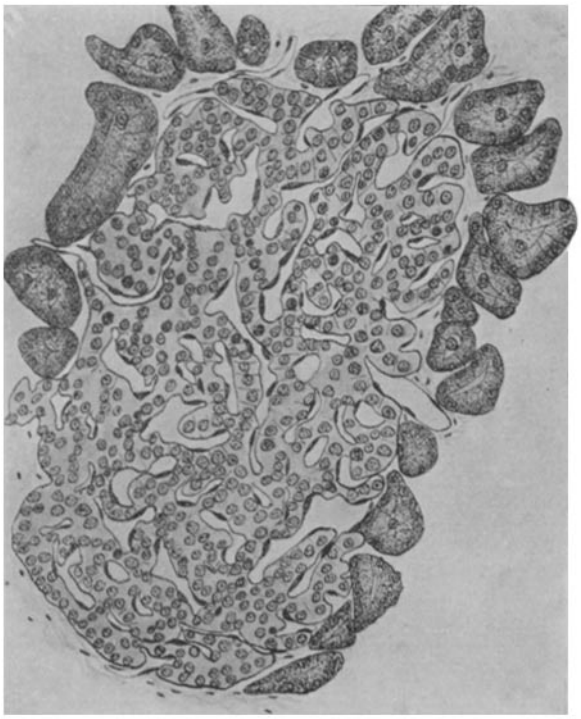


Fig. 3.—Section through central part of area of Langerhans from pancreas of rat. × 200.

In this series of sections, there was passive congestion, so that all veins and venous connections were filled with blood, while the arteries were as a rule empty. It was therefore an especially favorable series of sections for determining the relation of the vascular supply to the pancreatic vessels. As stated before,

v. Ebner and Diamare regarded the vessels of the areas as venous, while Laguesse, Opie, Kühne and Lea, Pensa and others believed it to be arterial, and Hansemann finds only capillary connections. After a most careful investigation of this series of sections as well as of many others from this and many other species, I have never been able to demonstrate an arterial connection with the vessels of the areas, since I have not found a vessel with distinct muscular coat either entering the areas or sending branches into them. Sometimes an arteriole was seen apparently approaching an area, but on tracing it through the series of sections, it was seen to turn aside or pass over the area without communicating with it. In cases of venous congestion, the blood-vessels of the islands are always packed with blood, and in a few cases, as in Fig. 4, I have been able to trace the main vessel, which have regarded as afferent, directly to a vein, beside which ran an artery with distinct muscular coat. In double injections, as will be shown later, it is always the venous injection mass which fills the blood-vessels of the areas. I have therefore regarded the blood-vessels of the areas as venous with abundant capillary connections with the surrounding interacinar capillary plexus.

Nearly always in tracing areas through a series of sections, at least one and sometimes more than one larger vein, usually arising either from an interlobular or intralobular vein, has been seen to enter the area and this I have designated as the principal or afferent vein, while several smaller venules and large numbers of capillaries leave the area in all directions. Since all of these vessels have the same structure, it is difficult, except by the size, to determine which are afferent and which efferent, and we can say only that several larger veins and numerous capillaries form a rich plexus of large, irregular, thin-walled blood-vessels, which, in accordance with Minot's characterization, I regard as sinusoids. The insular vessels always have a different arrangement from that of the interacinar vessels, but the arrangement is variable, especially in sections. Since the areas of the rat are usually much longer than they are wide, the principal vessel usually enters at the side, often passing, as in Fig. 4, to near the center of the island and there breaking up into branches,

which divide and redivide, the secondary branches often communicating by means of capillaries. Since Fig. 4 represents a section only 5μ in thickness, some idea of the rapidity of the branching and the centralization of the larger branches may be gained, although a thicker section would show greater continuity of the vessels. In sections stained with hæmatoxylin and eosin, the endothelial walls of the blood-vessels seem to rest directly upon the cords of epithelial cells constituting the island, and in most places no connective-tissue capsule can be seen. Where the area reaches the periphery of the lobule as seen in Fig. 3 and as is quite common, especially with the larger areas in the central portion of the pancreas, loose connective tissue forms its boundary and occasionally nucleated connective tissue may be seen in other places separating the area from the acini. In sections stained with Mallory's connective-tissue stain, a delicate blue line is seen separating most of the cells; a thin, but nearly if not quite complete, capsule of connective tissue surrounds the area and sends in still more delicate sheaths for the blood-vessels.

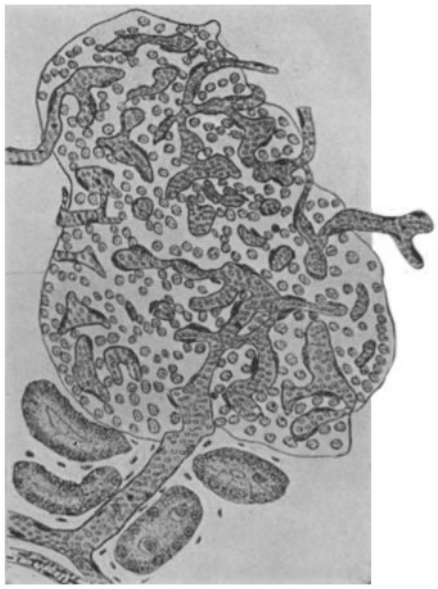


Fig. 4.—Section of area of Langerhans from pancreas of rat in which the veins were filled with blood. The figure shows the connection with the large vein and the arrangement of the sinusoids within the island as seen in a thin section. ×200.

In the *rabbit*, I have found on an average about one area for each square millimeter of section. As the average size of areas measured was .0034 c. mm., about $\frac{3}{1000}$ of the pancreas was insular tissue. The measurements taken were as follows·

1.	0.12 x 0.22 x 0.09 mm	0.0024 c. mm.
2.	0.15 x 0.23 x 0.15 mm	0.0052 c. mm.
3.	0.08 x 0.23 x 0.18 mm	0.0033 c. mm.
4.	0.12 x 0.18 x 0.10 mm	0.0021 c. mm.
5.	0.16 x 0.20 x 0.15 mm	0.0048 c. mm.
6.	0.13 x 0.16 x 0.18 mm	0.0037 c. mm.
7.	0.15 x 0.23 x 0.08 mm	0.0028 c. mm.
8.	0.10 x 0.11 x 0.095 mm	0.0011 c. mm.
9.	0.14 x 0.18 x 0.255 mm	0.0064 c. mm.
10.	0.15 x 0.15 x 0.10 mm	0.0023 c. mm.

A comparison of these measurements shows a considerable difference in the actual size of the areas, since Number 9 is nearly six times as large as Number 8. As may be seen from the measurements, as well as from Plate XII, Figs. 2 and 3, which reproduce two wax reconstructions of fairly typical islands from the pancreas of a rabbit, the islands are nearly always considerably longer in one dimension than in the other two. They are not, however, at all regularly oval, since there are numerous projections which run out between the pancreatic acini. The cells are of about the same types as described for the guinea-pig and rat, but in the sections observed by me there was an unusually large number of the large distinctly contoured eosinophile cells, with large, excentric, deeply staining nuclei. Such cells are more numerous near the periphery of the area. These often seem to merge into the acinal cells so gradually that no line of demarcation can be made out. In some places the lines of nuclei in the bands of cells pass out regularly into the cells of a longitudinally cut tubule which shows the typical basal position of the nuclei and the nuclear stain of the basal protoplasm, eosinophile granules being present in the central portion of the protoplasm. The same or similar appearances are not rarely seen in all forms in which the capsule is very thin and the relation to the acini very close. I have, however, regarded the two structures as separate unless, as frequently happens in the rabbit, the connection can be clearly traced through several sections of the series. This has been done in numerous instances in the rabbit and the connection may be seen in the models represented in Plate XII, Figs. 2 and 3, and also in the section shown in Fig. 5. This appearance can best be explained by the fact shown by Pearce and others that the areas develop from the same epithelial anlage as do the pancreatic tubules, and it may easily be

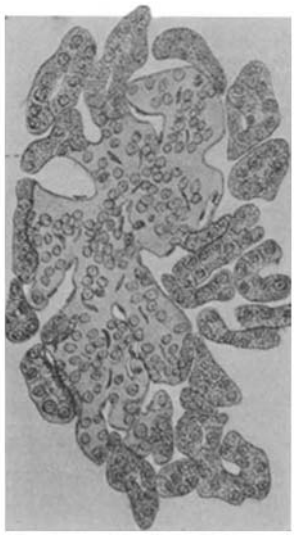


Fig. 5.—Section through the center of an area of Langerhans from pancreas of the rabbit; p, connection with pancreatic tubule. × 200.

that in the rabbit and other lower forms, as well as in some pathologic conditions in the human infant, the connection with the tubules remains unbroken. As seen in Fig. 5, the band-like arrangement of the cells is much less marked in the rabbit than in other species, the bands usually consisting of so many and so irregularly arranged cells that they may be spoken of rather as cell masses.

The character and arrangement of the blood-vessels and connective tissue are very similar to that already described for the guinea-pig and rat. Fig. 6 represents a curious anomaly, two examples of which were found in the sections of the rabbit's pancreas studied by me. These were not reconstructed, but were carefully traced through the entire series of sections in which the area appeared. They were nearly spherical masses of perfectly normal island tissue, which however formed merely a shell, the interior being filled with blood and having an endothelial lining. The occurrence of this blood vesicle or sinus, although only as an anomaly, may serve to lend some support to the idea that the circulation here is sinusoidal. Added interest is given to the anomaly by the fact that Laguesse describes in sheep embryos forms in which the island cells form hollow balls, the interior being filled with blood cells. A similar appearance (blood vesicle) in the petromyzon is mentioned by Pensa.

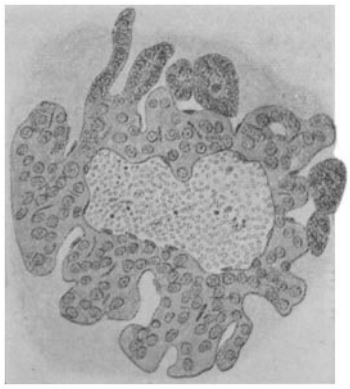


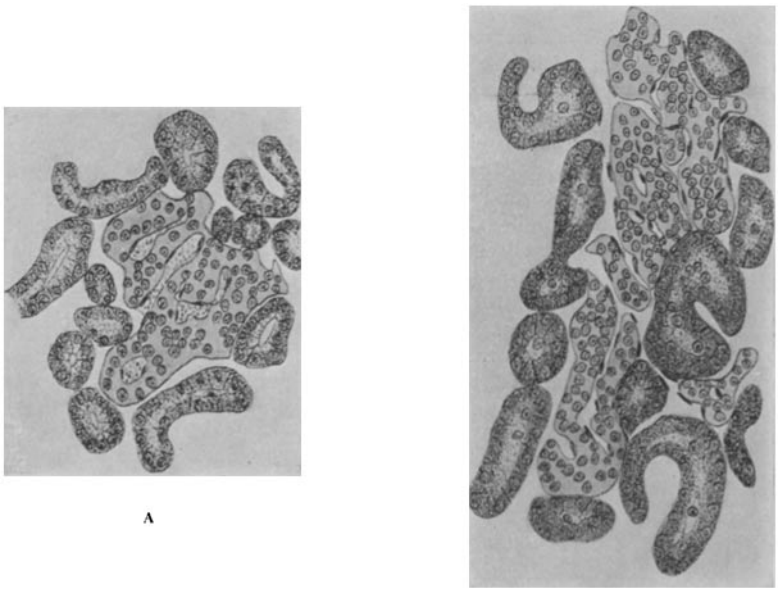
Fig. 6.—Section through center of a vesicular area from pancreas of the rabbit, the interior of the area being filled by a large blood sinus surrounded by endothelial cells which rest directly on a shell of normal island tissue. +200.

In the *cat*, the areas are as a rule much smaller than in the other forms studied. Twenty areas were measured, taken from different portions of the pancreas and from two different animals. The average size of all the areas was about 0.0027 c. mm. The average number per mm. was 1.08, so that about $\frac{3}{1000}$ of the pancreas consisted of island tissue.

	Cat I		Cat II	
1.	.08 x .105 x .18 mm.	.0015 c. mm.	12 x .16 x .11 mm.	.0021 c. mm
2.	.14 x .19 x .125 mm.	.0033 c. mm.	.11 x .17 x .15 mm.	.0028 c. mm.
3.	.12 x .25 x .10 mm.	.003 c. mm.	.19 x .32 x .135 mm.	.0082 c. mm
4.	08 x .17 x .09 mm.	.0012 c. mm.	.07 x ,20 x .13 mm.	.0018 c. mm.
5.	.09 x .18 x .14 mm.	.0023 c. mm.	.12 x .19 x .20 mm.	.0046 c. mm.
6.	.11 x .13 x .13 mm.	.0019 c. mm.	.13 x .25 x .125 mm.	.0041 c. mm.
7.	.08 x .14 x .14 mm.	.0016 c. mm.	.11 x .20 x .135 mm.	.003 c. mm.
8.	.11 x .18 x .11 mm.	.0022 c. mm.	.14 x .17 x .12 mm.	.0029 c. mm.
9.	.14 x .16 x .20 mm.	.0045 c. mm.	.20 x .22 x .125 mm.	.0024 c. mm.
10.	.10 x .14 x .15 mm.	.0021 c. mm.	.12 x .17 x .12 mm.	.0028 c. mm.
	Average size	.0024 c. mm.	Average size	.0034 c. mm.

It will be seen that the average size in the second series is greater

than in the first and that the areas are generally more nearly spherical or oval than in certain of the other mammals observed, though many of them are considerably elongated, as seen in Fig. 7, B. In Plate XIII, Figs. 1 and 2 are reproduced wax reconstructions of two small areas of Langerhans from the pancreas of an adult cat. These appear, as a rule, unless the sinuses are distended with blood or injected, as nearly solid masses of cells, most of the capillaries being entirely collapsed and seen only as a line of endothelial cells. Especially on the surface are the vascular openings small, so that the cellular elements much predominate. The varieties of cells are in large part the same as those already described; the cells are arranged in irregular masses and to some extent in bands consisting of a single or double row of cells. While the arrangement is variable and all possible forms are seen, it is not unusual to see the cords with the intervening sinusoids passing entirely or nearly across the area, as seen to some extent in both A and B of Fig. 7.



A B

Fig. 7. A and B. Central sections of two areas of Langerhans from pancreas of cat. × 200.

A number of double injections of the pancreas of the cat were attempted and while the results were not so successful as could be desired, in certain portions of the tissue the injection was such that arteries containing the red granular mass could be found, by the side of which could be traced a vein containing the blue gelatin mass and from there could be traced the principal branches passing to a Langerhans area. Certain of the sections from this tissue were cut relatively thick, so that one section would often contain the whole or nearly the whole of some of the smaller areas, and thus it was possible

to get in sections a very good idea of the arrangement of the sinusoids and capillaries in the islands. In Plate XIII, Figs. 3, 4, 5, and 6, are reproduced models reconstructed from thin sections of these injected areas, the blood-vessels only being used in the reconstruction. Three main types of arrangement of vessels may be noted —(1) The vessel runs along the side, either outside or just within the margin, of the area and sends branches mainly from one side into the area; the branches cross the area, branching and winding through it in every direction, and being often connected by anastomosing capillaries. (2) One or two main branches pass through the center sending branches in every direction. (3) The main branch reaches the center of the area and there breaks up into branches which radiate in every direction.

In the models, the arrangement can be quite easily traced in the smaller islands of Langerhans, but in the larger, the arrangement becomes so complicated that tracing is difficult, and the figure can give only a very incomplete idea of the arrangement. In the small islands, there is usually one main (probably afferent) vein, which passes to one of the larger veins, either interlobular or intralobular. Plate XIII, Fig. 3, shows a small and simple area in which the arrangement of the blood-vessels may be easily traced. A relatively large vein runs along the side of the island and sends off a short principal afferent vein. This breaks up almost at once into four smaller vessels, three of which may be seen in the figure. After winding through the area and branching and anastomosing several times, five vessels leave the area, two being capillary in size and the others larger. In Plate XIII, Fig. 4, the main afferent vessel divides, soon after entering the island, into five main branches which branch and anastomose many times, the blood leaving the area by one large efferent vein and six capillaries. The island represented in Plate XIII, Fig. 5, is composed of a plexus consisting of the branchings and anastomoses of two larger vessels and twelve capillaries, while the still larger area represented in Plate XIII, Fig. 6, shows a plexus composed of four larger vessels and fifteen capillaries with their branches. Two of the large vessels form an S-shaped curve and again meet to form the core of the area, the other branches and capillaries twisting around and between them to make up the intricate vascular plexus seen in the figure.

The *human areas of Langerhans* seem much more variable in structure than those found in the other species studied, age and the general condition of the body being more important factors in their structure than appears to be the case in most of the other vertebrates. This is especially true as concerns the insular connective tissue. I have counted and measured areas of Langerhans (1) from a four-year-old child (possibly syphilitic), (2) from a new-born, normal, healthy infant, and (3) from an apparently normal human adult. The measurements are as follows:

	I		II	
1.	.21 x .22 x .28 mm.	.0129 c. mm.	.15 x .17 x .13 mm.	.0033 c. mm.
2.	.20 x .28 x .35 mm.	.0196 c. mm.	.20 x .21 x .18 mm.	.0076 c. mm.
3.	.23 x .26 x .30 mm.	.0179 c. mm.	.16 x .18 x .20 mm.	.0058 c. mm.
4.	.16 x .25 x .20 mm.	.008 c. mm.	.15 x .15 x .17 mm.	.0038 c. mm.
5.	.15 x .17 x .23 mm.	.0059 c. mm.	.14 x .15 x .18 mm.	.0037 c. mm.
6.	.23 x .28 x .42 mm.	.027 c. mm.	.13 x .15 x .14 mm.	.0027 c. mm.

7.	.16	x	.225	x	.27	mm.	.0097	c. mm.	.16	x	.19	x	.21	mm.	.0064 c. mm.
8.	.19	x	.20	x	.265	mm.	.01	c. mm.	.12	x	.14	x	.17	mm.	.0029 c. mm.
9.	.18	x	.22	x	.225	mm.	.0089	c. mm.	.17	x	.23	x	.15	mm.	.0059 c. mm.
10.	.24	x	.28	x	.29	mm.	.0194	c. mm.	.12	x	.21	x	.17	mm.	.0043 c. mm.
	Average size						.0139	c. mm.	Average size						.0047 c. mm.

III

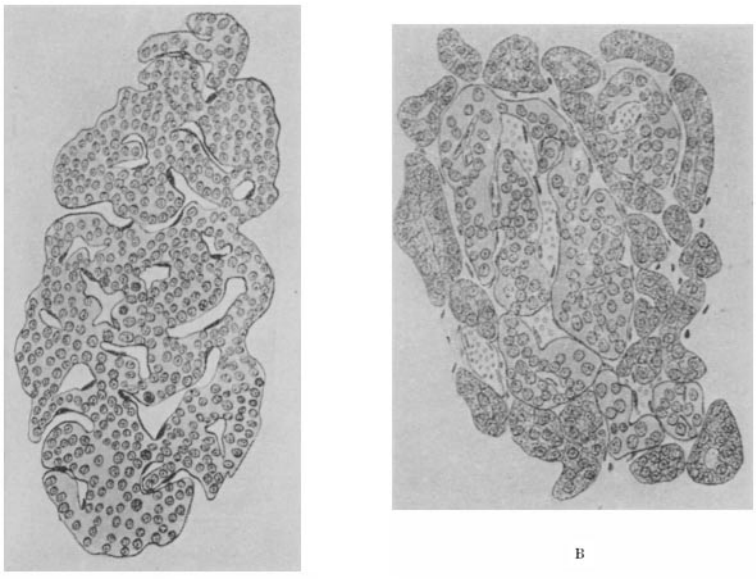
1.	.25	x	.35	x	.325	mm.	.0284	c. mm.
2.	.23	x	.24	x	.225	mm.	.0124	c. mm.
3.	.20	x	.38	x	.225	mm.	.0171	c. mm.
4.	.23	x	.33	x	.225	mm.	.017	c. mm.
5.	.18	x	.20	x	.16	mm.	.0057	c. mm
6.	.24	x	.25	x	.215	mm.	.0129	c. mm.
7.	.24	x	.30	x	.32	mm.	.023	c. mm.
8.	.17	x	.23	x	.275	mm.	.0108	c. mm.
9.	.23	x	.34	x	.25	mm.	.0195	c. mm.
10.	.15	x	.20	x	.215	mm.	.0065	c. mm.
	Average size						0.0153	c. mm.

In Series I of the table, the number of areas averaged $3\frac{1}{7}$ to each c. mm. of the section, so that about $\frac{4}{100}$ of these sections was area tissue. In Series II, the average number was only 1.3 to each c. mm. of the section and hence only about $\frac{8}{1000}$ of this pancreas was island tissue. The average number of areas in each sq.mm. of the adult pancreas (Series III) is 1.5, making about $\frac{2}{100}$ of the pancreas consist of insular tissue. My figures are slightly different from those given by Opie, Laguesse, and others, since Laguesse finds that on the average only $\frac{1}{100}$ of the human pancreas is island tissue. This can be explained only as an individual variation, since in the figures of the observers mentioned there is quite as marked a variation between any two cases. It is worth noting that the average size in the adult is actually greater than in either of the other cases, while in the four-year-old child it is greater than in the newborn infant. This is quite different from the results frequently reported, since it is usually stated that both the size and the number of islands of Langerhans diminish with age, being greatest in the fœtus and new-born. In the four-year-old child, however, the number in a sq. mm. was greater than in either of the others, and the proportion of area to glandular tissue was much greater in this than in either of the others.

The shape of these areas varies greatly, but is in the main ovoid, though the contour is quite irregular. Something of the shape and general appearance may be seen in Plate XIV, Figs. 1 and 2. Fig. 1 reproduces a wax reconstruction of a fairly typical area from the adult human pancreas, while Fig. 2 is the negative of Fig. I, representing the strands of connective tissue with blood-vessels, etc. Since there was some shrinkage of the epithelial cells, the strands are relatively somewhat larger than they should be, but otherwise indicate the arrangement of the blood-vessels in the larger and more complicated areas like the human. Plate XV, Figs. 1 and 2, reproduce the interior view of the models represented in Plate XIV. The looser and more open appearance of the central portion of the areas is readily seen in these figures. It may also be seen that in some regions two and in others three larger sinusoids run through the area in

either direction, so that we find much the same appearance in whatever direction the sections are cut. These large sinusoids are connected at intervals by smaller vessels and, as the greater part of the island is composed of superposed layers of similar arrangement, it may easily be seen why so large a number of sections through the larger portion of the area show a very similar arrangement of cords of cells and blood-vessels. The rich branching of the larger vessels and the connections of the smaller ones may also be seen in the figure.

Since the arrangement of the blood-vessels regulates the arrangement of the bands and groups of cells, a description of the one figure necessarily involves a description of the other. The resemblance of the cords of cells to tubules and acini appears more marked in the interior of the model than in the exterior view. It may be repeated, however, that not only is no lumen found, but there is not even any arrangement of cells which would suggest a lumen. The cells are most irregularly grouped, and the only suggestion of regularity of arrangement seems to be due to their following the arrangement of the blood-vessels. The cells of the human areas vary in size and structure and may be divided into about the same classes as were described for the guinea-pig. The amount and arrangement of the insular connective tissue vary more than any other portion of the structure. In the child's pancreas, there is very little or no connective tissue within the island, the sinusoids resting directly upon the epithelial cells. A space may often be seen between the capillary wall and the



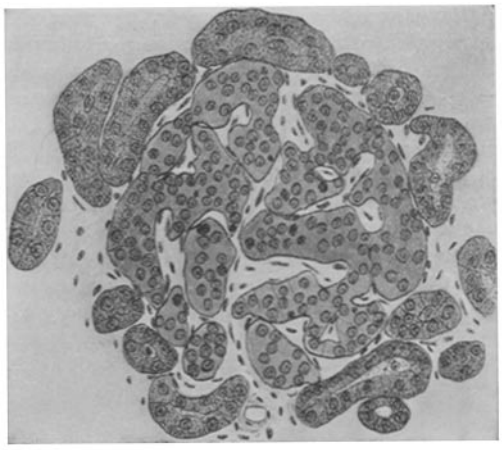
A B

Fig. 8, A and B.—Sections of areas of Langerhans from pancreas of four-year-old child. × 200.

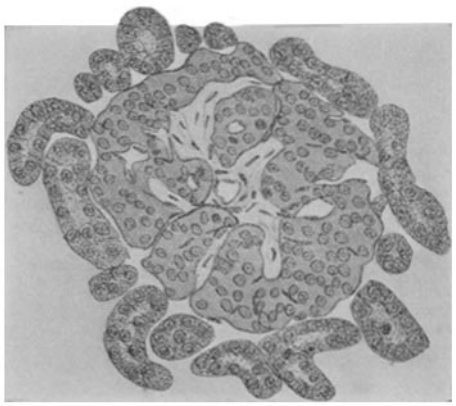
epithelial cells, indicating that the cells have shrunken somewhat either in the fixation or as a result of post-mortem change. No such spaces were observed

in the fresh pancreas of the new-born infant. No connective tissue can be made out in the pancreas of the four-year-old child with the ordinary stains, but with Mallory's stain a delicate capsule surrounding the area and delicate sheaths around the blood sinusoids can be made out.

In the pancreas of the new-born infant, nearly all the areas observed were found in the interlobular connective tissue and surrounded by it, but no connective tissue could be made out within the areas. In Fig. 8 are reproduced sections of two insulæ from the pancreas of the four-year-old child. The cells are somewhat smaller, the nuclei appearing smaller and more crowded than in the islands of the adult pancreas. This is still more the case in the areas of the younger child. In Fig. 8, B shows an area which has irregular projections into the acinal tissue, as if it had been somewhat broken up by the ingrowth of acini. It is evidently not so near the center of the island as A, which represents a very typical section. In the adult, at least in most of the islands seen by me, there is more connective tissue, forming rather definite trabeculæ, which divide the island into smaller compartments containing the cells; this recalls the description given by Harris and Gow, who, because of this tendency, speak of the human areas of Langerhans as compound. Sometimes the connective tissue forms, with the larger blood-vessels, one or many larger trabeculæ passing through the center, other septa passing to the sides, much as described and figured by Flint. Such an area is shown in Fig. 9, A, showing the central strand cut longitudinally, while in B, it appears to be cut transversely, giving the radiating



A



B

Fig. 9, A and B.—Central sections through two areas of Langerhans of the human pancreas. × 200.

arrangement of the secondary strands, similar to that described for the blood-vessels of the bird. Such differences may be readily understood from a reference to Plate XV, Fig. 2, if we imagine a section cut through the large sinusoids which run nearly parallel to the plane of the section in that figure, we will have much

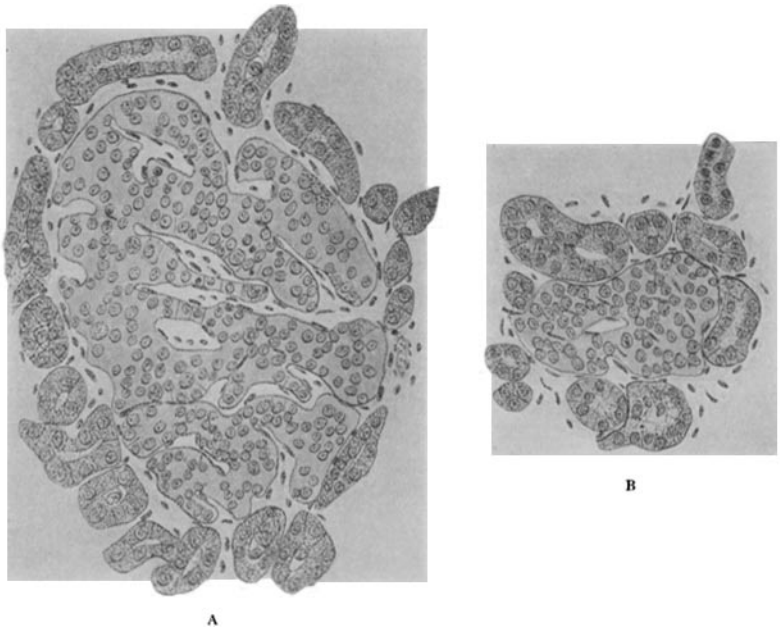


Fig. 10, A and B.—Sections of areas of Langerhans from pancreas of human adult. A, near center of area; B, near periphery. × 200.

the appearance given in Fig. 9, A. If the section is cut in the same direction, but between two layers of the larger sinusoids, we will get more the appearance given in Fig. 9, B. In general, however, the strands of connective tissue in the human areas, like the sinusoids of other forms, have a very irregular arrangement, scarcely any two islands presenting exactly the same appearance.

A rather definite capsule of nucleated connective tissue surrounds most of the areas. The only thing which is constantly noted, in the child as well as in the adult, is that in the interior of the islands the strands of connective tissue as well as the blood-vessels contained in them are larger and the masses of cells smaller and more band-like, while the individual cells are generally larger than at the periphery. This makes it easy, regardless of the size of the section, to distinguish a central from a peripheral section. Fig. 10 may serve to illustrate this point, A, of this figure, as well as A and B of Fig. 9; representing sections from near the center of an area, while Fig. 10, B, represents a section near the periphery. The increase of connective tissue with age or with general sclerosis of the gland and the very small amount in younger individuals and in animals seem to indicate that the formation of the connective tissue within the islands is secondary.

In birds, the areas of Langerhans have been found by Mouret, Harris and Gow, Pugnát, Diamare, and others. Diamare asserts that after careful study of numerous serial sections, he is able to affirm that this structure in birds is entirely similar to that in other vertebrates—"the islands consist of very vascular epithelial cords." Pugnát studied the pancreas in many species of birds, but described it especially in the dove. He states that the pancreas consists of three lobes, each with a distinct duct and that the cells do not form acini, but rather a reticular structure like the liver. The islands, he states, are large and irregular, consisting of small, elongated cells with indistinct protoplasm and faintly staining nuclei. Both Mouret and Pugnát regard them as lymphoid structures. I have examined the areas of Langerhans in the dove and in the goose and found them similar, but because of the greater distinctness of the islands in my sections from the dove, my descriptions and figures will be taken from this animal.

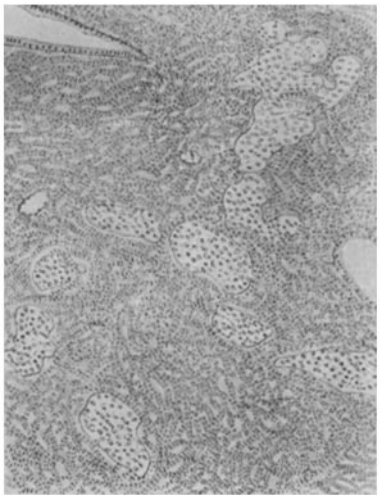


Fig. 11.—Portion of section through central part of dorsal pancreas of dove. × 50.

In the *dove* the two main lobes of the pancreas are found n the loop of the duodenum, one ventral and one dorsal and separated by the mesentery. In the ventral lobe, the areas of Langerhans are few, small and distinct. In the dorsal lobe, on the other hand, they are large and prominent. and it is from this lobe that my figures are taken. The pancreas was congested and all the blood-vessels of the areas were filled with blood and hence were very distinct. It is difficult, on account of the diffuse character of these areas, to determine with any exactness their size or number. They are larger and more numerous near the central part of the lobe than at the periphery. Fig. 11, a low-power drawing of a small portion of this part of the dorsal pancreas, may serve to show the size and irregularity of shape and apparent number of the islands in this region.

Several of these, however, unite into one area in a later section, so that the

number is smaller and the size greater than is apparent in any one section. The lighter areas represent the islands of Langerhans, and the darker portion the glandular t ssue which I may say in passing seems to me to form definite tubules and acini, as in the pancreas of other vertebrates and not, as stated by Pugnát, a recticular stucture like the liver, although under low power it does present a reticular appearance.

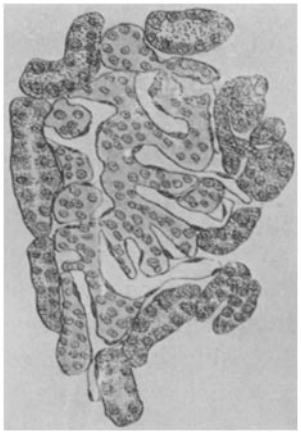
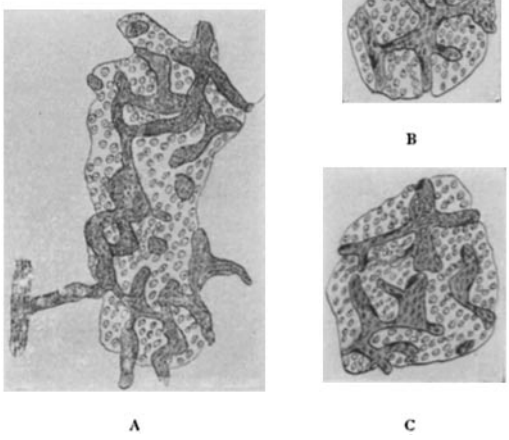


Fig. 12.—Section through central portion of dorsal pancreas of dove. × 200.

The cells of the bird's islands are smaller and more uniform in size and structure than those found in the islands of any other vertebrate examined, scarcely any variation in size or stain of nuclei being noted. The cell outlines can scarcely be made out. The nuclei are small, round, or more often oval, with a very distinct nucleolus. Both nucleus and protoplasm stain faintly. The cells are arranged in definite cords between the sinusoids, which here are wide and prominent because of the congestion. Fig. 12 reproduces a central section of an area of Langerhans from the pancreas of the dove, the blood cells in the sinusoids being omitted. Fig. 13 reproduces three areas of Langerhans from the pancreas of the dove, in which the blood-filled sinusoids are especially emphasized, the cells appearing lighter in the figure. They show three types of arrangement: A shows the origin from a larger vein of the main afferent vessel, which enters the island, passing along one side and dividing into two main branches near the end. Most of the other branches are given off from one side of the main sinusoid, these again dividing and passing in different directions. A large branch is given off from the main sinusoid, which passes back, winding partly under the latter, and supplies the proximal portion of the area This is



A B C

Fig. 13, A, B, C.—Three sections of physiologically injected areas of Langerhans of dove, the injected sinusoids being especially emphasized in the figure. × 200.

a relatively thin section, so that many of the tortuous capillaries are discontinuous, but it indicates very well the connections and arrangement of the insular vessels. B shows the main sinusoid passing through the center of the area and sending branches to either side, while C represents a radial branching from a central large sinusoid. C might, as will be readily seen, represent a transverse section of B.

In the frog, the islands stain more deeply and are so diffusely and irregularly scattered among the pancreatic tubules that counting and measuring are very difficult. It is almost impossible to determine how many of the small island masses seen among the acini belong to a single area or to be certain as to the limits of the areas. The most of the area sections seen by me were relatively small, averaging .002 c. mm. in size, the dimensions averaging about 90 μ x 120 μ x 210 μ. The cells differ somewhat in character from those in the other areas studied, since very tall columnar cells with elongated, deeply stained nuclei predominate. These are crowded together, usually in a single row, especially in the central portion to form the very distinct cell bands so characteristic for amphibia; they resemble those described and figured by Diamare for the triton. He states that "the small epithelial cords are rather delicate, formed of rather slender cells, closely approximated and are separated by large capillaries. These last evidently correspond to the 'large venous sinuses' of v. Ebner. No capsule separates the areas from the zymogenic tissue." Pensa also in speaking of the cylindric cells of the islands of amphibia, says: "These cells are not aggregated into cords but are arranged in linear series, one after the other in single file." In addition to these narrow crowded cells which make up the central cords, masses of polygonal cells with spherical nuclei are usually found, especially at the periphery of the islands. V. Ebner described these islands in the frog as early as 1872, stating only that they showed no lumen penetrable by a mass injected into the duct and that rather large veins are found near the islands. In his later communication in 1899 he states that he regards the vessels of the areas of Langerhans in the frog as venous.

Fig. 14 represents a very typical area from the pancreas of the *frog*, with its tall, narrow columnar cells with oblong, deeply stained nuclei arranged in

rather regular bands. Polyhedral cells with round or oval nuclei staining more faintly are scattered irregularly between the columnar cells and form more definite masses at the periphery. The sinusoids are distinct with simple endothelial lining and no connective tissue demonstrable, either surrounding the area or around the intrainsular sinusoids.

I have made no study of fish and reptiles, but Diamare and Harris and Gow and others have described large insulæ in reptilia, and Diamare and Rennie and others have found similar structures in fishes, the islands in these forms having the same structure as in the types studied by me; certain of the very large ones are, however, constant in position and, in some species of fish, are independent of the glandular tissue of the pancreas.

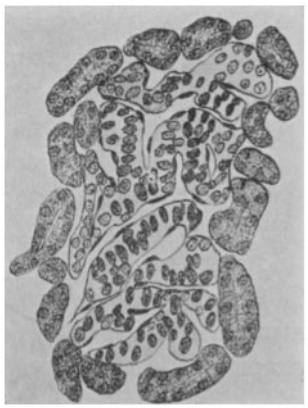


Fig. 14.—Section of area of Langerhans from pancreas of frog. × 200.

In general, then, it may be said that areas of Langerhans of very similar structure are found in all species of vertebrates that have been examined. In all species, they consist of cords or masses of epithelial cells derived from the same anlage as the pancreatic acini and sometimes retaining their connection with the glandular tubules. The cells vary somewhat in type in any one area, but the same or similar types are found in all the species examined, except the frog and bird; in the bird the cells are uniformly small and generally oblong, both nucleus and protoplasm staining poorly; in the frog, the predominant cells are tall and columnar with long deeply stained nuclei, the cells being packed together in single rows separated by blood-vessels. In all cases, the cords of cells are separated by large, irregular, anastomosing blood-vessels, having a complete endothelial wall and no or very little adventitia. In most forms, the endothelium seems to rest directly on the epithelial cells, and the connective tissue, when present, appears to be a secondary development, as in the somewhat sclerotic, adult human pancreas. The vessels correspond to Minot's definition of sinusoids. The

vascular network of the islands is made up of the windings, branchings, and anastomoses of several larger vessels of venous origin and large numbers of capillaries, which communicate intimately with the interacinar capillary plexus. The largest sinusoids are found in the central part of the islands, where the cords of cells are relatively small. The periphery of the areas is generally much less vascular. The largest areas found in my series were those of the rat, those of the guinea-pig being second. The reconstructions show that the cords of cells have the external form of branching and anastomosing tubules with occasional alveolus-like enlargements ; they are, however, solid structures with no lumen and no arrangement of the cells and nuclei which would suggest a lumen.

The areas are generally larger and more numerous in the central portion of the pancreas, especially in the bird, guinea-pig, and rat. In most species, the islands are intimately related to the glandular tissue, the capsule, when present, being so thin and delicate that it is demonstrated with difficulty. In the newborn child, however, most of the areas lie in the interlobular connective tissue and are surrounded by it; in the guinea-pig, the largest areas are in the connective tissue surrounding the larger ducts and are independent or practically independent of the pancreatic acini; and in some species of fish certain of the islands are very large, constant in location, and entirely independent of pancreatic tissue. In the human accessory pancreas, islands may or may not be present, the number reported in which they were absent being approximately equal to that in which they were present.

It is very natural to inquire concerning the function and meaning of an organ so constant in its presence and structure at all ages and in all species, occurring so early in embryonic life, and maintaining its vitality with so few changes through all conditions, and it is this side of the question which has interested the great majority of the investigators of the islands of Langerhans. The most varied views prevail. The discoverer, Langerhans, suggested the possibility of a relation to the nervous system, since these structures are often found in close proximity to the sym-

pathetic ganglia and the related nerves and staining reaction of their cells in ordinary dyes is not dissimilar. Because they are found in embryonic life and appear larger and more numerous in the embryo and new-born than in the adult, Gibbes, Harris and Gow, and others, regarded them as embryonal remains. Krause, Kühne and Lea, Renaut, Sokoloff, Dieckhoff, Pischinger, Pugnát, Mouret, and others basing their theory on the character of the cells, regarded them as lymphoid structures. Dogiel and Tschassownikow considered them as exhausted acini, or as Dogiel states "todte Punkte" because he found no connection with the duct and found fat globules in the cells.

Lewaschew, Laguesse, Mankowski, Statkewitsch, Pischinger, Saviotti, Kollossow, and many others assert that the islands are merely temporarily changed acini, which may change back into pancreatic acini. The reasons for this view are well expressed by Mankowski. He states that the number of islands increases during the period of gland activity and diminishes during rest; that the islands of Langerhans represent one of the morphological stages of activity of the pancreas. Every lobule of the pancreas must, at the end of its period of active secretion, pass into the "Stadium der Langerhans'schen Inseln," which represents the morphological phenomena of the greatest exhaustion or the greatest activity. He also notes the occurrence in any one section of various stages of transition between gland acini and the insulæ. Finally Zunz, Ssobolew, von Ebner, Schultze, Jarotzky, Sauerbeck, Diamare, and probably the great majority of the very recent writers on the subject regard the islands as independent organs, vascular glands, arising from the same anlage as the pancreas, but having a different function,—the elaboration of a secretion which is poured into the blood. This theory is based partly upon clinical and partly upon experimental data and will be considered more in detail later.

If perfectly fresh and well-fixed pancreas is examined, the cells of the islands, with their relatively small nuclei and prominent protoplasm and their arrangement into definite bands separated by sinusoids, are sharply differentiated from adenoid tissue, which can sometimes be seen in close proximity. The develop-

ment of the areas from an epithelial anlage also speaks against the theory of the lymphatic structure. That they are not embryonal remains may be readily seen from the fact that they do not in any sense degenerate in adult life, but remain alive, with a rich blood supply, and are, according to my experience, only relatively larger and more numerous in the young than in the adult. The rich blood supply, the absence of any appearance of degeneration, and the occurrence of dividing cells, as noted by Bizzozero and Vassale, by Schulze, and as I myself have seen, all refute the theory that they are pancreatic acini undergoing regressive changes. These dividing cells were especially numerous in the islands of the young child's pancreas, but some cells undergoing division were seen in nearly every section stained in Heidenhain's iron-lac-hæmatoxylin. The theory that the areas are being constantly derived from pancreatic acini and changing again into the glandular tissue is based (1), on the increase or diminution in the number and size of the areas during different conditions of digestion, and (2), on the occurrence of transitional forms, especially as shown by Lewaschew, after repeated pilocarpinization. Opie and Hansemann have repeated Lewaschew's experiments with pilocarpin, making careful counts of the islands, but have found no increase in the number of islands and no transitional forms. Ssobolew tested hunger, active digestion, and pilocarpin and found areas only slightly altered in number.

There is no question that structures do appear at times which might be interpreted as transitional forms. I have not infrequently seen groups of tubules in which the characteristic differentiation into two zones was entirely absent, the nuclei being centrally placed in the cells; these groups looked not unlike large islands of Langerhans, especially if, as sometimes happens, no lumen could be made out in the tubules. Sometimes, also, the eosinophile cells of the areas may be so numerous and so arranged as to resemble somewhat the tubules just mentioned. In the guinea-pig, I have once or twice seen, in apparent connection with one of the central areas, tubules having no outer basophile zone and having the nuclei centrally placed. These,

however, were very rare, and by tracing them through the series of sections, I was generally able to convince myself that they were merely contiguous with the areas and not continuous. The appearance of the pancreatic tubules varies so much under different conditions, and the relation of the tubules to the islands is, in most animals, so intimate, that it would not be difficult in almost any section to find structures which might be regarded as transitional. I have seen none, however, which could not be explained in some other and more rational way.

As to the size and number of the islands in different stages of digestion and under different diet conditions, Mankowski says that the areas increase during digestion, while Hansemann states that the apparent increase is due only to a more marked differentiation because of the change in the acini themselves.

To test the *changes produced in the islands of Langerhans by digestion and diet*, I have examined, measured, and counted a large number of islands from a considerable number of guinea-pigs under the following conditions: (1) normal in full digestion; (2) after different periods of time without food or drink; (3) after they had been kept on pure carbohydrate diet for different periods; (4) after the same periods on pure meat diet. As the guinea-pigs had to be forced to the meat diet, the earlier animals showed purely the changes of inanition, and I have not included them in my table. In order to make the conditions as nearly parallel as possible, I, in all cases except the first, killed the animal about fourteen hours after eating, divided the fresh pancreas into three approximately equal portions, fixed them in saturated bichloride solution, and cut the sections longitudinally in the direction of the main duct and through that portion of the gland which included the main duct. The sections therefore represented approximately the same regions of all the organs examined. I have measured sections of areas rather than the three dimensions, since the work previously reported has been based on the appearances noted in sections. The variations in size due to cutting through peripheral portions of the islands were overcome by measuring large numbers and taking the average. I counted and measured all the islands in the entire section in each case and in at least five sections, so that my average may certainly represent a true average for the areas in this region of the pancreas. While some qualitative changes were noted in the areas,—such as an increase or diminution of the number of eosinophile cells, a granular change in the cells, atrophy of cells with increase of intercellular substance,—there were none which could be regarded as constant for any one experiment and constantly increasing with the duration of the experiment. The table given below gives a brief synopsis of the main data gained from the counts and measurements. The largest number of very large islands was seen in the animal which had been kept on a meat diet for sixty days, while the proportion of island tissue to pancreatic glandular tissue is

constantly greater under meat diet than under carbohydrate diet or in hunger. But since, as may be seen from the measurements given of the normal areas, there are marked individual differences even under the same circumstances, I believe that this variation is due to individual peculiarities, although Ssobolew states that the size and number of the areas are quite constant for each species. In the following table, 1, in each case, represents the splenic third of the pancreas, 2, the middle third, and 3, the end nearest the duodenum.

Experiment.	Av. no. per mm.	Av. size in sq. mm.	Proportion of gland to island.	Size of largest area in sq. mm.	Size of smallest area in sq. mm.
Normal (full digestion)					
1.	.35	.02	1: .007	.31 x .38	.04 x .06
2.	.42	.014	1: .006	.20 x .21	.04 x .06
3.	.68	.021	1: .014	.21 x .43	.05 x .06
Hunger–36 hours					
1.	1.54	.012	1: .018	.13 x .37	.04 x .05
2.	1.22	.0064	1: .008	.19 x .20	.03 x .04
3.	.71	.014	1: .01	.24 x .36	.03 x .05
Hunger–46 hours					
1.	.346	.023	1: .008	.40 x .54	.02 x .04
2.	.4	.014	1: .015	.15 x .20	.03 x 11
3.	.18	.036	1: .006	.25 x .48	.05 x .06
Hunger–52 hours					
1.	.46	.018	1: .008	.19 x .52	.02 x .04
2.	.52	.015	1: .008	.19 x .23	.05 x .06
3.	.51	.009	1: .005	. 3 x .18	.03 x .04
Carbohydrate diet 30 days					
1.	.39	.018	1: .006	.18 x .33	.0 x .08
2.	.74	.013	1: .009	.38 x .40	.04 x .05
3.	.43	.019	1: .008	.20 x .35	.06 x . 07
Carbohydrate diet 60 days					
1.	.39	.016	1: .006	.19 x .21	.06 x .08
2.	.5	.019	1: .0099	.22 x .34	.05 x .06
3.	.52	.019	1: .0098	.18 x .50	.06 x .07
Meat diet, 30 days					
1.	.75	.015	1: .011	.13 x .50	.03 x .05
2.	.69	.015	1: .0102	.26 x .40	.05 x .06
3.	1.01	.012	1: .012	.17 x .19	.04 x .05
Meat diet, 60 days					
1.	.57	.032	1: .018	.30 x .68	.05 x .07
2.	.54	.025	1: .013	.27 x .42	.05 x .07
3.	.4	.031	1: .012	.22 x .68	.06 x .08

The so-called transition forms are merely resting pancreatic tubules. The size and number of the islands have individual variations under normal conditions fully as great as occur under varied conditions of digestion, hunger, and diet, and no changes occur under these conditions as has been shown by Opie, Schulze, Diamare, Jarotzky, and by myself. That the protoplasm of the insular cells, as Mankowski himself states, is chemically different

from that of the pancreatic cells is shown by the marked differences in their affinity for stains, especially for silver nitrate and safranin. As has been shown by many experimenters, the islands may be well preserved when the gland tissue is extremely atrophied, so that they do not seem to be subject to the same conditions as pancreatic cells. Of especial importance is the fact that islands have been found in some species independent or practically independent of the pancreas. For these reasons we certainly seem justified in the conclusion that the islands of Langerhans are independent organs with an independent function.

For over a century, cases of diabetes have been reported in which the pancreas was diseased, and the tendency has been constantly increasing to regard pancreatic disease as one, at least, of the causes of diabetes. This tendency has been greatly augmented since 1889, when Minkowski showed that in dogs the removal of the pancreas gave rise to glycosuria and most of the symptoms accompanying diabetes in man. Since then, medical literature has abounded with clinical and pathological reports as well as with chemical and experimental studies on the relation between the pancreas and sugar metabolism. After lesions of the islands of Langerhans in association with diabetes mellitus were described by Opie in 1900, discussion has been limited to the relation between sugar metabolism and the islands of Langerhans. I do not intend to enter in detail into the pathological reports, since my own work does not to any extent deal with the clinical side of the question and since pathologists are still at variance in their conclusions. Suffice it to say that Opie, Weichselbaum and Stangl, Wright and Joslin, Herzog, Ssobolew, and many others believe themselves justified in accepting the theory that pancreatic diabetes is due to some disturbance of the function of the areas of Langerhans, while Hansemann, Gutmann, Reitmann, Karakaschew, Herxheimer, Dieckhoff, Benda, and others contend that this theory does not explain and can not be based upon the facts observed.

Recently Sauerbeck has collected from the literature most of the published cases of diabetes in which the condition of the islands was reported and has added to them a number of his

own cases. He states that 117 of the 157 cases of the series show abnormal islands of Langerhans. In seven cases the islands could not be found. In one, they were represented by scar tissue. The changes described are as follows: (1) a diminution in size and number; (2) qualitative changes, such as hæmorrhage, fatty degeneration, acute and chronic inflammation, simple atrophy, hydropic degeneration (Weichselbaum and Stangl), sclerosis, and hyaline degeneration. If we reject as too indeterminate the purely quantitative changes, we still have 98 of the 157 cases in which qualitative changes were observed. Most of these changes have, however, in greater or less degree, been reported in non-diabetic cases, and eleven cases have been described by Dieckhoff, Hansemann, Litten, and Ziehl, in which the pancreas was entirely destroyed by suppuration or by carcinoma and yet no diabetes occurred, while non-diabetic cases have been observed in which no normal islands were found. Whether it be that in the complexity of function of the human organs, some other organ or tissue takes up the work of the areas under certain conditions, whereas in other cases they fail to do so, cannot be stated with any certainty. Several writers have asserted an interrelation of function between the pancreas, the liver, and the spleen, while Lorand has recently asserted that he has proved by experiments on dogs a relation between the function of the areas of Langerhans and the thyroid. He therefore states that the areas of Langerhans secrete a substance which neutralizes the poison produced by the thyroid and that diabetes may result either from the increased functional activity of the thyroid or from diminished activity of the areas of Langerhans. However this may be, all that can be said in the present condition of our knowledge is that the anatomo-pathologic investigation of this question has not as yet led to any satisfactory solution.

The announcement of the relation of the pancreas to sugar metabolism gave an impetus to an investigation of this question from the chemical point of view. Arnheim and Rosenbaum asserted the presence of a glycolytic substance in pancreas, in muscle, and in liver, the glycolysis being much increased if pancre-

atic extract were mixed with either the muscle or the liver extract. Šimáček claims to have isolated from the pancreas by precipitation with alcohol and ether a substance which, under anaërobic and aseptic conditions, causes an energetic alcoholic fermentation. Feinschmidt also obtains a glycolytic substance from liver, muscle, and pancreas, while Stoklasa has found a similar substance in plants and in numerous organs and fluids of the animal body, and Croftan claims to have found a glycolytic substance which he calls trypsin in the human blood. Stoklasa, Šimáček, and Feinschmidt find as the products of the glycolysis alcohol, carbon-dioxide, and acids. Cohnheim, however, claiming that this alcoholic fermentation is the result of bacterial influence, has published a series of articles in which he shows that there is present in muscle a glycolytic ferment, which, however, is inactive until acted upon by a substance in the pancreas which he calls an "activator." This substance is not destroyed by boiling and is soluble in water and alcohol but insoluble in ether. Increasing the amount of the pancreatic extract or of the ether precipitate of the same increases the influence up to a certain point, beyond which any further increase diminishes the glycolytic action. He regards this action as analogous to that of Ehrlich's amboceptor and complement. The presence of blood in the muscle causes glycolysis without addition of pancreatic extract and sometimes the glycolysis is diminished when pancreas is added. This fact would indicate the presence in the blood of variable amounts of the activator principle of the pancreas. He concludes that this substance is not a ferment since it is not destroyed by boiling, but is analogous to the other products of internal secretion, adrenalin, iodothyrin, and secretin. Fichera examined liver, muscle, cartilage, and epithelium of depancreatized dogs and found that the glycogen diminished and finally disappeared in about thirty days after the operation and concludes that the main phenomena of diabetes are a diminution of normal amylogenesis and a weakening and often disappearance of the glycolytic functions of the organs.

As has already been mentioned, in 1889 Minkowski succeeded in totally extirpating the pancreas of dogs, the operation being

followed by glycosuria and other symptoms of diabetes. Since then the operation has been repeated on dogs, cats, rabbits, birds, frogs, turtles, and eels, by numerous investigators, among whom may be mentioned de Dominicis, Minkowski, Harley, Kausch, Weintraud, Aldehoff, and Marcusi. While the results obtained by different investigators have varied somewhat, owing no doubt to the different methods used in the operation and also in the subsequent urinalysis, glycosuria following pancreas extirpation has been found in all the species examined, with the exception of ducks and geese, in which Kausch was able to demonstrate a hyperglycæmia but no glycosuria. De Dominicis found glycosuria in only about two thirds of the seventy animals (dogs, cats, rabbits, pigs, etc.) upon which he operated, but in all there were some changes such as polyuria, polyphagia, polydipsia, azoturia, phosphaturia, etc. Glycosuria was affected somewhat by diet, drugs, etc. Lüthje and others assert that the removal of the pancreas does not entirely destroy the function of glycolysis, since they found that the sugar disappeared from the urine while it was still present in the blood. Lépine and Thiroloix state that diabetes does not follow extirpation of the pancreas, if the animal is starved for some days before the operation. Pflüger, in order to test the constancy and permanency of glycosuria, removed the pancreas in a considerable number of animals, being careful that the extirpation should be total. He found that in every case glycosuria resulted and led to the death of the animal. He noted also that the liver always increased in size with an increase in fat, but that glycogen was absent.

After partial extirpation of the pancreas, the results are more variable and depend, as Minkowski thought, not so much on the size of the portion of the pancreas that is left as on its condition. The same seems to be true in most cases whether the remnant is left in situ or is transplanted under the skin, provided the blood supply is not interfered with.

In 1891, Vassale first noted the preservation of the islands and the glandular atrophy resulting from ligation and section of the pancreatic duct in rabbits. In 1898, Katz and Winkler found the islands of Langerhans preserved for some time after

the ligation of the duct. In 1900, Schulze tied off small portions of the pancreas in guinea-pigs, causing a complete atrophy of the ligated glandular portion, which was replaced by connective tissue, while the islands were unaffected. After eighty days he finds mainly connective tissue with a few dilated ducts and the normal areas of Langerhans. Since no glycosuria resulted from his experiments, he drew the conclusion that the areas of Langerhans are vascular glands of the type of the hypophysis, having an internal secretion whose function is probably to regulate the sugar content of the blood. In 1901, Mankowski repeated Schulze's experiment, tying two ligatures, and examining the portion between the ligatures, as well as the portion on either side of the ligatures. His results and conclusions were directly opposed to those of Schulze. He found both areas of Langerhans and pancreatic tubules atrophic and sclerosed as a result of his operation; as he notes an increase in the number of islands during digestion and a diminution in the resting stage of the pancreas, he decides that the islands are merely temporarily changed acini.

In 1902, Ssobolew ligated the duct and divided it between the two ligatures in dogs, cats, and rabbits. In cats, he found that the ducts generally reunited and became permeable before any extreme atrophy could take place. He found rabbits most favorable, since the duct could be tied and cut without injury to the pancreas. In the rabbit's pancreas, he found, as did Schulze in the guinea-pig, that the gland tissue became atrophic while the areas were well preserved up to the four hundredth day, at which time his observations ceased. At that time, the gland consisted only of the normal areas, surrounded by connective tissue containing the main duct. He describes the following changes in the glandular tissue: (1) There is loss of zymogen granules, protoplasm is homogeneous, and nuclei are irregular, shrivelled, and deeply stained. (2) Cells disappear and membrana propria collapses. (3) Centro-acinar cells are not seen after the eleventh day. (4) The changes in the glandular cells consist of atrophy, vacuolization, granular change, and sometimes indirect cell division. (5) Cells of small ducts increase and to some

extent also those of the medium-sized ducts. (6) Between the fourth and fifteenth days, there are mucous degeneration of cells of large ducts and thickening of walls. (7) After the thirtieth day there are atrophy of small ducts, collapse of membrane, and degeneration of epithelium. (8) New formation of connective tissue is interlobular, and there is much new elastic tissue around ducts and islands. (9) The number of blood-vessels diminishes after twenty days, the number of nerves after from sixty to seventy days; the number of ganglia and of cells in the remaining ganglia diminish and Vater-Pacinian bodies degenerate and do not reappear. (10) If measures were not taken to prevent it, there was a new formation of excretory ducts through which the secretion could be carried to the duodenum. Pawlow and Smirnow and Tiberti also note this regeneration of the duct and with it of the gland tissue, if the degeneration has not gone too far. (11) As to the areas, Ssobolew makes an important statement. While he finds the areas normal in the rabbit four hundred days after the operation, he finds that earlier, between the thirtieth and one hundred and twentieth day, some of the islands degenerate and many are reduced in size, but that there are individual differences in the power of resistance.

In none of the animals operated upon, did Ssobolew find glycosuria, but he does not state how frequently he examined the urine or how long after the operation he continued to do so. The point is of especial importance since Hédon in 1894 noted a slight transient glycosuria from the first to the fourth month after the pancreatic duct was filled with oil, thus causing atrophy of the gland. Sauerbeck (1904) calls attention to the same fact and also to its significance. He states that in rabbits (the same species on which both Hédon and Ssobolew operated) he noticed after ligation of the duct a slight glycosuria developing after the first month and at the same time a distinct alteration of the islands. This coincidence of facts is extremely suggestive for the island theory of diabetes.

Zunz (1905) after ligation of the excretory duct of the dog's pancreas noted the same atrophy of the gland parenchyma with preservation of the areas as described by Schulze, Ssobolew, and

Sauerbeck. Hansemann (1902), however, after ligating the duct in ten dogs, states that the atrophy extends only a short distance beyond the ligature and that the gland is later restored to a considerable extent. The islands may be well preserved or they may undergo fibrous degeneration. Lombroso (1905) tied and cut the duct in dogs and pigeons and found the glandular parenchyma practically unchanged. He states also that the islands show changes proportionate to those in the glandular tissue. It would seem to me probable that the inconsistent results obtained by these two investigators are due either to incomplete obstruction of the duct or to the regeneration of the duct which is so commonly met with, since their operations seem to have had so little effect on either the structure or the function of the pancreas. Lépine (1905) comes to the conclusion that the acini, as well as the areas of Langerhans, take part in furnishing the internal secretion to the blood and bases his conclusion on the following facts: (1) in some diabetics the insulæ are affected and in others, the glandular tissue; (2) after ligation of the pancreatic duct or injection of oil into it, the glycolytic power of the blood is greatly increased. As interference with the outflow of the secretion can affect only the gland parenchyma and not the islands, he considers this as sufficient proof that the acini as well as the islands form the glycolytic principle which is given to the blood.

Since the experiments of Schulze and Ssobolew and others showed that by ligation of the duct and interference with the outflow of the pancreatic secretion, a complete atrophy of the glandular acini of the pancreas was caused, while the areas of Langerhans remained unchanged, it seemed possible that by their method the areas of Langerhans might be isolated in considerable portions of the pancreas and the physiologic action of their extract determined; it was with this purpose that my experiments were undertaken. My experiments were performed in great part upon cats. Three were attempted upon guinea-pigs, but these were unsuccessful, as the pigs died during the first three days after the operation. In order to obtain as large an amount of extract as was consistent with the life and well-being of the operated

animal, I endeavored to pass my ligature as close as possible to the point of union of the two ducts. This point varies somewhat in different individuals, but in the main is about as represented in Fig. 15. In this figure, the ducts had been exposed

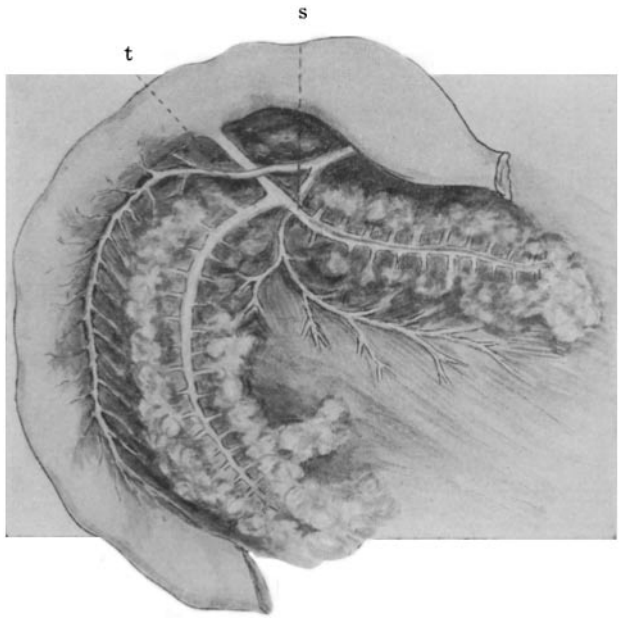


Fig. 15. Pancreas of cat laid open to show main ducts and their relation to the large vein near the junction of the ducts. The letters are explained in the text.

by dissection throughout their entire length, having been first filled with a blue solution. The blood-vessels had been previously injected with a red mass. The two halves of the pancreas were then laid back to expose the duct. The delineator has not represented quite accurately the relative size and length of the two portions of the gland, but the point of junction of the ducts and the relation of the ducts to the blood-vessels at the point of operation are nearly correct. My ligature, then, in five operations was at *t;* five glands were ligatured at *s* and *t*, and two at *s* only; ten had two ligatures tied tightly in the neighborhood of *s*, the pancreas being completely divided between the ligatures and the severed ends thoroughly cauterized to prevent the re-

union of the duct which had apparently taken place when the gland had been simply ligatured. In seven cases, after dividing the gland between two ligatures at *s*, a fold of omentum was drawn between the two ends and sewed or tied in place.

The animals lived after the operation from eighteen hours to 197 days. Ten of them, on account of an acute inflammation (three cases) or inanition (the cats often refused all food for the first week or ten days after the operation), died too early to hope for a sufficient degeneration of the gland tissue to attain even an approximate isolation of the islands. The changes found in the glandular parenchyma were much the same as those described by Ssobolew. The larger ducts were much dilated and filled with desquamated epithelial cells and detritus. The smaller ducts and the acini could scarcely be differentiated, since the gland cells had in the main early lost their differential staining power, the outer zone no longer taking the basic stain nor the inner the acid stain, but both staining alike. The cells in both small ducts and acini in many cases disappeared. The membranæ propriæ then collapsed and appeared like strands of connective tissue. The interlobular connective tissue was greatly increased, so that the lobules were much compressed, but still retained the appearance of lobules. In few, if any, of my cases has the lobule been entirely replaced by connective tissue except in the immediate neighborhood of the ligature, where often both gland tissue and areas of Langerhans had completely disappeared to give place to new connective tissue. This was especially the case in those animals in which the gland was divided and cauterized.

In my earlier operations, no special effort was made to avoid including the blood-vessel in the ligature and in some of these cases islands as well as gland tissue had suffered atrophy. In the other experiments, in which an effort was made to avoid interference with the blood supply of the gland, the areas were quite well preserved regardless of the extent of atrophy of the gland tissue. In those animals killed during the first six weeks or two months, however, many of the islands appeared smaller and less numerous than normal, although no accurate counts

and measurements were made. I at first considered this apparent atrophy as due to the poorer differentiation caused by the changes in the acinar cells, but they may represent the earlier and transitory atrophic changes noted by Sauerbeck, Ssobolew, and others, especially as in those animals that lived more than sixty days the areas appeared perfectly normal.

After some time, in some of my cases the duct became permeable again, as shown by passing a colored fluid through it into the duodenum, and in one case the enlarged and sclerosed duct broke through to the surface of the pancreas and so found vent for the secretion. In either case, there was a partial regeneration of the glandular tissue, and the regenerated tubules seemed to originate from the old, collapsed tubules that made up the atrophied lobule. The cells, though non-granular, flattened, non-functionating, and appearing like connective-tissue cells, had not lost the power of functionating and soon regained the appearance of secreting acini. In such glands, shown especially well in two experiments (No. XVI, 94 days and No. XXIII, 197 days), only two or three tubules with cells having granular protoplasm could be seen in a lobule section generally toward the center where the pressure of the connective tissue is less than in the outer part of the lobule, and where the rich blood supply of the areas of Langerhans may have helped to maintain the vitality of the cells. It was sometimes possible to trace a direct transition from the flattened and collapsed membrana propria which appeared merely as strands of connective tissue, through strands of cells containing a few granules to tubules staining like normal pancreatic acini. This easily and naturally explains the rapid regeneration of an extremely atrophied gland when an exit is made for its secretion. It may be to this method of regeneration that Ssobolew referred when he stated that in cats the ducts regenerated and the gland then soon regenerated, if the degeneration had not gone too far.

In most of the cases, I examined the urine during the first few days after the operation and again just before death. Sugar was not found in any of these cases, and as Sauerbeck's observations had not at that time appeared and I was unaware

of the possible occurrence of glycosuria after the first month, I did not continue the examinations long enough to be able to speak upon this point. In four of my cases, I tried the Mayo-Robson test on the urine just before death, with positive results in three cases and negative in one.

In all cases except when spontaneous death occurred during the night, the pancreas was removed immediately after the death of the animal and small pieces taken, one from the ligatured end, one from the neighborhood of the ligature, and one from the free end, and fixed in corrosive-sublimate solution for microscopic study. The rest of the gland, usually in two portions, was extracted either with glycerine or with water and its digestive and glycolytic powers tested. Starch digestion was tested with iodin and with Fehling's solution. Fibrin digestion was determined by the use of the bromine tryptophan reaction and the biuret test. Fat digestion was indicated by the reaction and by emulsification. The glycolytic power was tested by following as nearly as possible Cohnheim's method: a definite amount of glucose was added to a definite amount of muscle extract, and to this were added the pancreatic extract and also large amounts of toluol for its bactericidal action. In some cases, both boiled and unboiled pancreatic extracts, were used with the muscle extract and with the same results. The quantity of sugar was estimated, at once after mixing and again after twenty-four hours in the warm oven, with the ammoniacal copper solution of Pavy. Control tests with muscle alone and with pancreatic extract alone were carried on at the same time.

The results of these various *tests* are briefly indicated in the following table in which O indicates the mental end (usually the ligatured end), while D indicates the duodenal end (the free and hencemore normal end), of the pancreas. It will be noted from this table that seven of the twenty cases in which physiologic tests were made (and this included six of the ten cases in which the gland tissue was nearly normal) showed no digestion of starch, fibrin, or fat, while in several others the digestive action was very much weakened. The diminution and absence of digestive action are proportionate to the atrophic condition of the glandular tissue. In none of the cases, however, was there any appreciable weakening of the glycolytic or activator power of this extract, even when the glandular tissue was apparently atrophied

No.	Operation	Time	Glandular Parenchyma	Areas of Langerhans	Physiologic Tests				Other Organs.
					Starch	Fibrin	Fat	Sugar	
1	Ligature and section at *s.*	18 hrs.	Normal	Normal	Not tested				
2	Ligature, section, cauterization and omental insertion at *s.*	3 days	Hemorrhage near ligature	Normal	O. Pos D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
3	Three ligatures.	5 days	Vacuolation and compression of acini	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
4	Same as No. 2	6 days	Dilatation of ducts; compression of acini	Normal	No tests made				
5	Ligature and section.	6 days	No examination		No tests made				
6	Two ligatures at *s.*	7 days	Dilatation of duct; compression of acini	Small and poorly differentiated	No tests made				
7	Ligature, division, and cauterization.	8 days	Ducts dilated. Hemorrhage near ligature	Small; cells small and protoplasm degenerated	O. Pos D Pos.	Pos. Pos	Pos. Pos.	Pos. Pos.	
8	Ligatures at *s.* and *t.*	9 days	Vacuolation and compression of acini; necrosis and inflammation	Areas normal but small	No tests made				Liver; granular degen.
9	Ligature and section of duct at *s.*	10 days	Congestion and dilatation of ducts; acini compressed	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	Normal
10	Ligature, section, cauterization and insertion of omentum at *s.*	13 days	Hemorrhage near ligature	Normal	No tests made				Fat necrosis and hemorrhage
11	Ligature at *t.*	15 days	Atrophy and sclerosis of ligatured end	Some normal; some atrophied and sclerotic	No tests made				Kidneys; cloudy swelling, congestion and hyalin casts; liver: congestion, granular degeneration
12	Ligature, section, cauterization, and insertion of omentum at *s.*	16 days	Atrophy	Normal	No tests made				
13 14	Ligature, section, and cauterization at *s.*	27 days	Atrophy and sclerosis; dilatation of ducts	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	Normal
15	Ligature, section, cauterization, insertion of omentum at *s.*	49 days	Extreme atrophy and sclerosis	Normal; seem increased	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	
16	Ligature at *t.*	35 days	Atrophy and sclerosis; dilatation of duct	Normal	O. Pos. D. Neg.	Pos. Neg.	Pos. Neg.	Pos. Pos.	
17	Ligature, section, cauterization, insertion of omentum at *s.*	59 days	Extreme atrophy and sclerosis; dilatation of duct	Normal except near ligature	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	
18	Ligature and section at *s.*	87 days	Selerosis but little atrophy	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
19	Ligature at *t.*	90 days	Nearly normal	Normal	Pos.	Pos.	Pos.	Pos.	
20	Ligature, section and cauterization at *s.*	94 days	Extreme atrophy and selcrosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	

No.	Operation	Time	Glandular Parenchyma	Areas of Langerhans	Physiologic Tests.				Other Organs.
					Starch	Fibrin	Fat	Sugar	
21	Ligature, section, and cauterization at *s*.	100 days	Extreme atrophy and sclerosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	Liver: fatty degeneration
22	Ligature at *t*.	112 days	Slight sclerosis and atrophy	Normal	Pos.	Pos.	Pos.	Pos.	Liver: granular and fatty
23	Ligature at *s*.	114 days	Extreme atrophy and sclerosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	
24	Ligatures at *s*. and *t*.	114 days	Regeneration; atrophy and sclerosis near ligature	Normal	No tests made				
25	Ligature at *s*	119 days	Atrophy and sclerosis	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
26	Ligatures at *s* and *t*.	126 days	Atrophy and sclerosis	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
27	Ligature and section at *s*.	156 days	Atrophy and sclerosis	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
28	Ligature section, and cauterization at *s*.	197 days	Extreme atrophy and sclerosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	

The following table of the glycolytic tests in the seven cases in which there was no digestive action will indicate the very slight change, if any, in the activator power of the pancreatic juice caused by the extreme atrophy of the pancreatic acini. The slight difference is probably due to the smaller amount of the "activator principle" used in the case of the atrophied portion of the gland. (O, indicates omental and D, duodenal end of pancreas.)

No.	Time.	Original amount of sugar in grm.	Amount of sugar after 24 hours in grm.	Actual loss in grm.	Per cent. of loss.
15.	49 days.	O 2.0776 D 2.0776	1.315 1.209	0.762 0.868	.7% .8%
16.	53 days.	O 4.25 D 4.25	3.67 3.57	0.58 0.68	.6% .7%
17.	59 days.	O 1.66 D 1.66	0.83 0.81	0.83 0.83	.83% .81%
20.	94 days.	O 0.76 D 1.13	0.4 0.77	0.36 0.25	.12% .1%
21.	100 days.	O 1.0125 D 0.875	0.27 0.215	0.74 0.66	.8% .9%
23.	114 days.	O 0.69 D 0.93	0.21 0.29	0.48 0.64	.5% .6%
28.	197 days.	O 1.5 D 1.13	1.15 0.77	0.25 0.36	.41% .42%

The experiments have not proven so decisive as I had hoped, and for two reasons: (1) the isolation of the areas was not always

complete; and (2) the insulæ in the cat's pancreas are generally small and it is difficult to get, even from the larger omental portion of the pancreas, when atrophied, sufficient extract to make very many satisfactory tests. I feel sure, however, that the chemical and physiological examination of isolated island tissue offers the only certain means of deciding the difficult questions regarding the function of these structures. While my results must be regarded as relative rather than absolute, they speak with no uncertain voice in favor of the theory that the islands manufacture a substance, analogous to the "activator principle" described by Cohnheim, which favors the glycolytic action of muscle ferment. To make these results more certain, it is necessary to use the isolated island tissue in large enough amounts so that numerous tests may be made not only of its activator action on muscle ferment, but also its effect on blood pressure and, most important of all, its influence on the experimental diabetes of depancreatized animals and on human diabetics. It is a question whether the operative method can in any animal furnish the isolated island tissue in large enough amounts for these purposes.

Rennie, however, has recently described two species of fish (*Lophius piscatorius* and *Scorpæna scropha*) in which one large "principal islet" and several smaller islands of structure analogous to that of the areas of Langerhans in mammalia may be distinguished with the unaided eye and may be dissected out entirely free and isolated from pancreatic tissue. If these shall prove to have the same function as the areas of Langerhans in mammalia and other forms, they offer the opportunity for further and more satisfactory investigation along these lines. Since my experimental work was finished and had been reported in part at the session of the Association of American Anatomists at Philadelphia in 1904, two preliminary reports have appeared, one by Rennie himself and one by Diamare and Kuliabko, announcing that they are now following out a similar line of investigation on the isolated islands of these two species of fish. The results given in their preliminary reports are by no means decisive or very satisfactory. Rennie reports three tests, two

qualitative and one quantitative, on a glucose solution with an extract of these areas. The result would seem to show that this extract does not *alone* have the power of inverting grape sugar. He hints, however, that his experiments with this extract on diabetic patients have been more favorable to the island theory of diabetes. Diamare and Kuliabko, on the other hand, tested in several cases the action of this island extract on starch solution and on glucose solution. They were able to show, so far as their few tests made can decide, that these islands have no power whatever to digest starch. While the starch solution with the pancreatic extract from the same fish gave with iodine no starch reaction, and with Trommer's test gave marked sugar reaction, that with the island extract gave only the starch reaction with iodine. Their test for the glycolytic action is much less decisive, since no change was noted until after forty-eight hours, so that the inversion may have been due to bacterial action; further experiments, therefore, are necessary. The outline which they give of the work which they are now carrying on shows that they are carefully investigating the chemical and physiological characteristics of these isolated areas of Langerhans in the fish, and we shall hope for much light on this question when their work shall have been completed and their results published.

In conclusion it may be stated that there occur in the pancreas of all vertebrates homologous structures known as areas of Langerhans.

These have the structure of vascular glands with a sinusoidal circulation and are not changed during the secretory activity of the pancreas. They also remain unaltered when the glandular acini become atrophied as a result of ligation, section, or obstruction of the duct.

They have a secretion which is probably poured into the blood-vessels; this secretion has no digestive action on starch, fibrin, or fat, but has a marked glycolytic function, especially when added to muscle extract and is therefore analogous to the "activator principle" described by Cohnheim.

In closing I am glad to acknowledge my indebtedness to

Professor G. Carl Huber for many helpful suggestions received during the progress of this investigation.

EXPLANATION OF PLATES.

PLATE XII.

Fig. 1.—Wax reconstruction of typical island of Langerhans from pancreas of rat. × about 200.

Figs. 2 and 3.—Wax reconstructions of islands of Langerhans from pancreas of rabbit. P, connection with pancreatic tubules. × about 200.

PLATE XIII.

Figs. 1 and 2.—Wax reconstructions of areas of Langerhans from pancreas of cat. × about 200.

Figs. 3, 4, 5, and 6.—Wax reconstructions of injected blood-vessels of areas of Langerhans from pancreas of cat. × about 200.

PLATE XIV.

Fig. 1.—Wax reconstruction of area of Langerhans from human pancreas. × about 250.

Fig. 2.—Wax reconstruction of blood-vessels with surrounding connective tissue in same area, the cellular cords of which are reproduced in Fig. 1. × about 250.

PLATE XV.

Fig. 1.—Interior view of model reproduced in Fig. 1, Plate XIV, Fig. 1. × about 200.

Fig. 2.—Interior view of model reproduced in Fig. 2, Plate XIV, Fig. 2. × about 200.

BIBLIOGRAPHY.

Aldehoff.—*Zeit. f. Biol.*, 1892, xxviii, 293.

Blumenthal.—*Deutsche med. Woch.*, 1903, xxix, 961.

Cohnheim.—*Zeit. f. physiol. Chem.*, 1903, xxxix, 336; 1904, xlii, 401.

Croftan.—*Philadelphia Med. Jour.*, 1902, x, 71.

De Dominicis.—*Arch. ital. de biol.*, 1891, xvi, 28.

Diamare.—*Internat. Monatsschr. f. Anat. u. Phys.*, 1899, xvi, 155.

Diamare und Kuliabko.—*Zent. f. Phys.*, 1904, xviii, 14.

Dogiel.—*Arch. f. Anat. u. Phys.*, 1893, Anat. Abt., 117.

Fichera.—*Beiträge zur path. Anat. u. allg. Path.*, 1904, xxxvi, 273.

Flint.—*Arch. f. Anat. u. Phys.*, 1903, Anat. Abt., 61.

Gutmann.—*Virchow's Archiv*, 1904, clxxvii, Suppl. Heft., 128.

Hansemann.—*Zeit. f. klin. Med.*, 1894, xxvi, 191. *Verhandl. der deutschen path. Gesellsch.*, 1902, iv, 187.

Harris and Gow.—*Jour. of Phys.*, 1894, xv, 349.

Herxheimer.—*Verhandl. der deutschen path. Gesellsch.*, Ergänzungsheft; *Cent. f. path. Anat.*, 1904, xv, 215.

Fig. 1.

Fig. 2.

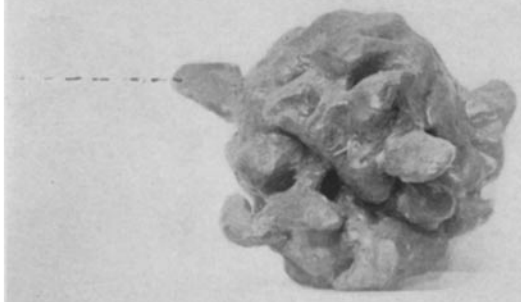


Fig. 3.

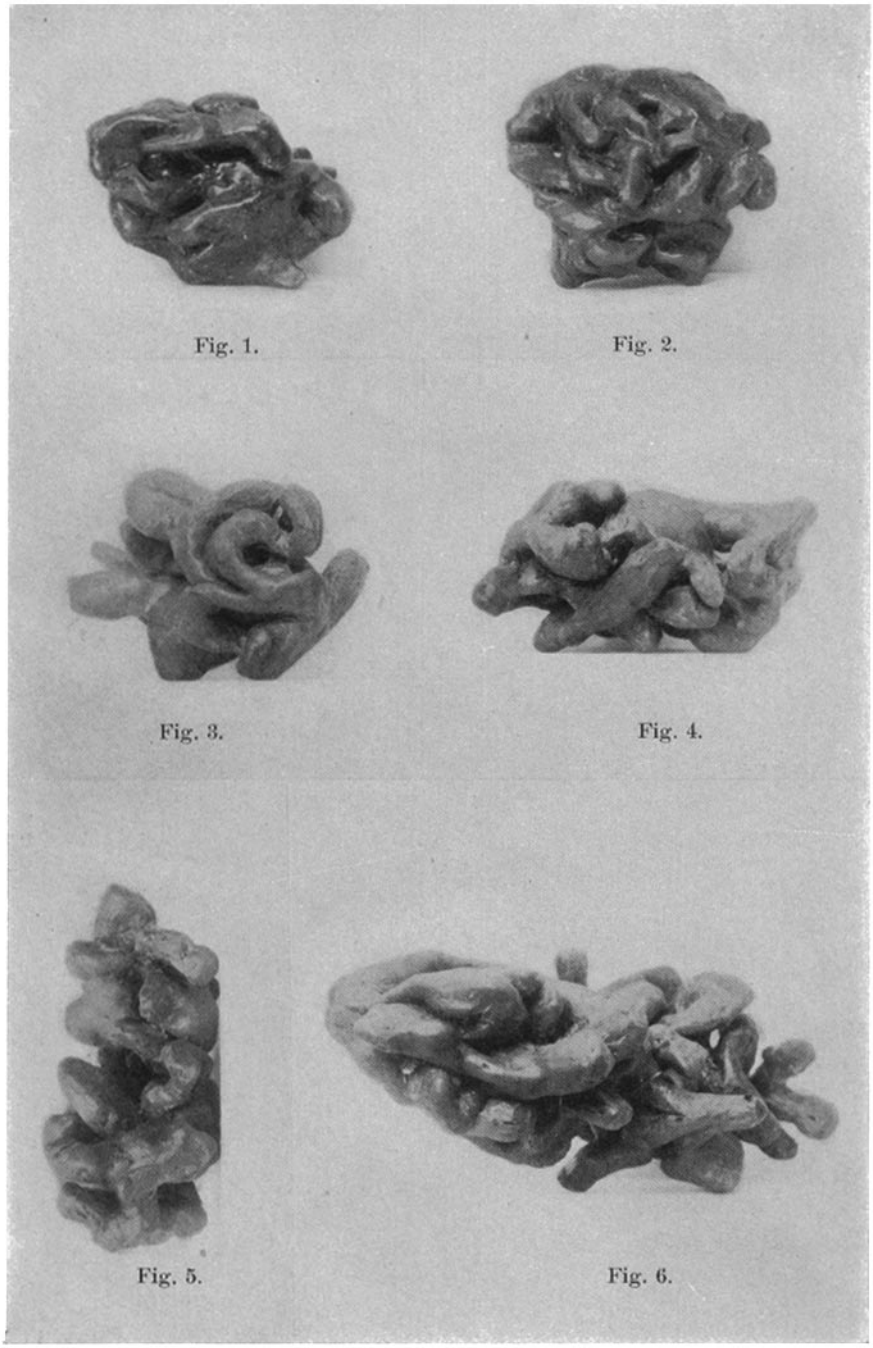


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

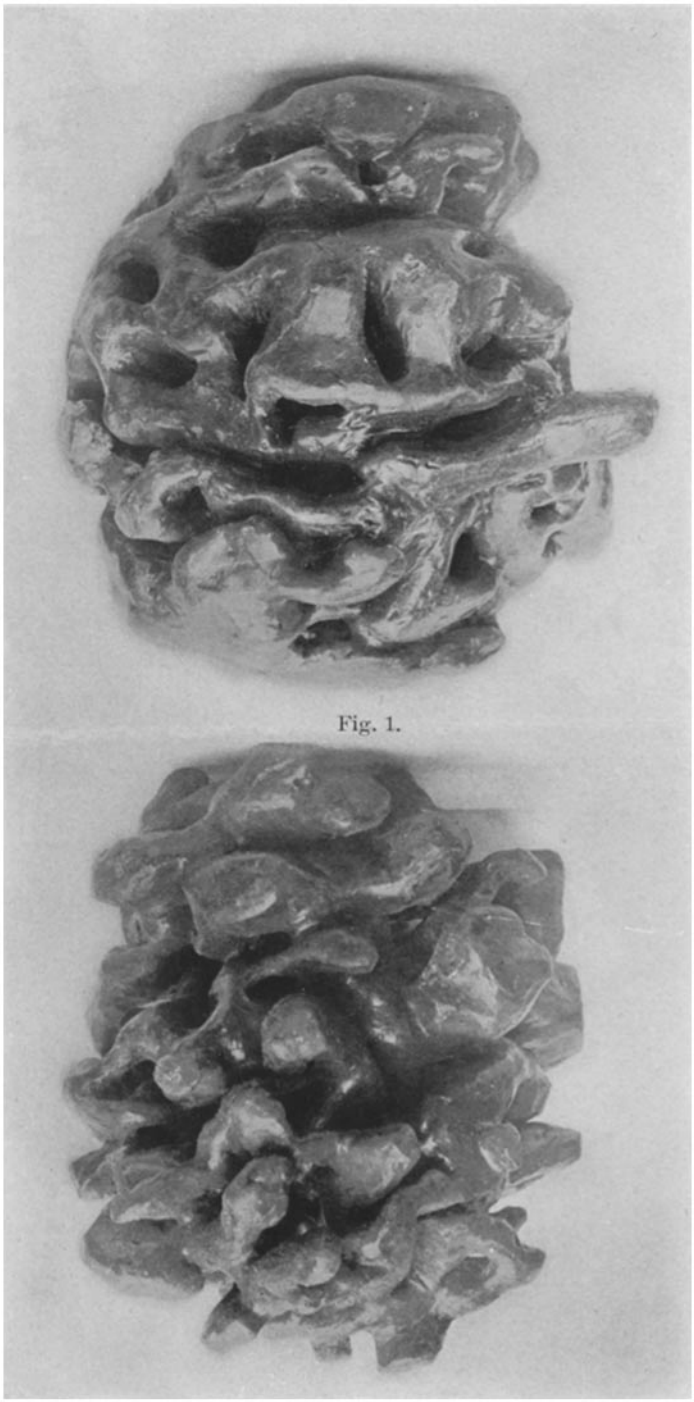


Fig. 1.

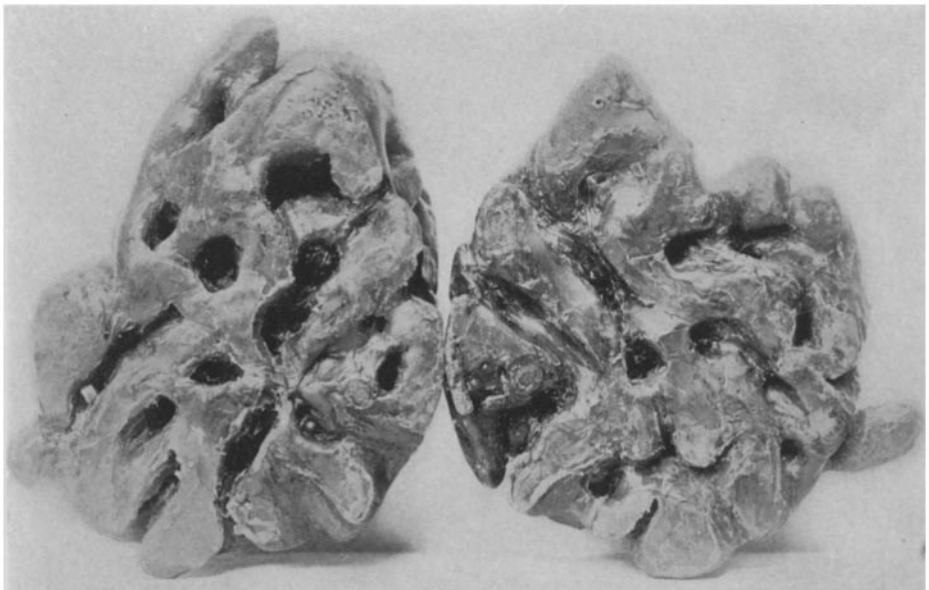


Fig. 1.

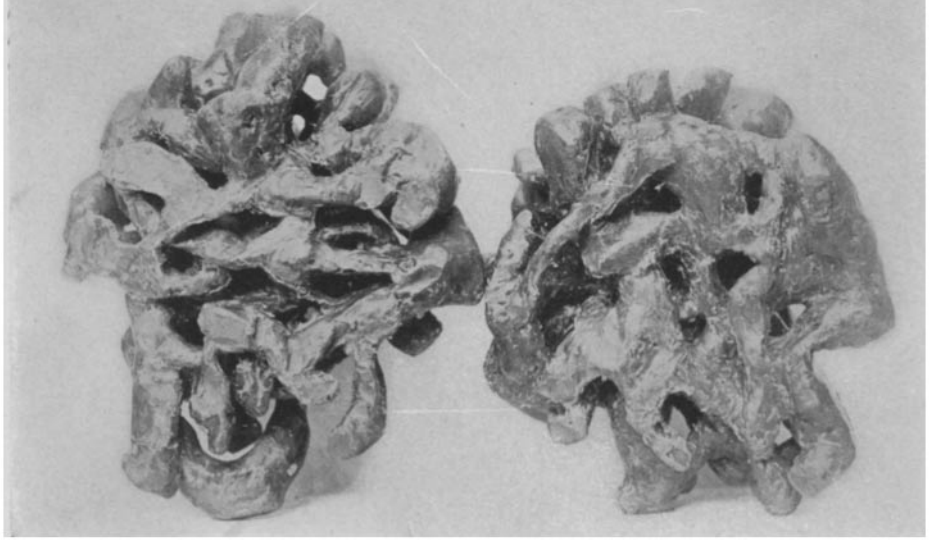


Fig. 2.

Herzog.—*Virchow's Archiv*, 1902, clxviii., 83; *Trans. of Chicago Path. Soc.*, 1901, v, 2.

Jarotzky.—*Virchow's Archiv.*, 1899, clvi, 409.

Karakascheff.—*Deutsches Arch. f. klin. Med.*, 1904, lxxxii, 60.

Kausch.—*Arch. f. exper. Path. u. Phar.*, 1896, xxxvii, 274.

Küster.—*Arch. f. mik. Anat.*, 1904, lxiv, 1.

Laguesse.—*Compt. rend. de la Soc. de Biol.*, 1894, xlvi, 667; *Jour. de l'anat.*, 1894, xxx, 591; *Compt. rend. de la Soc. de Biol.*, 1895, xlvii, 699; *Jour. de l'anat.*, 1896, xxxii; *Compt. rend. de la Soc. de Biol.*, 1905, lviii, 504; *ibid.*, 1905, viii, 542.

Lépine.—*Compt. rend. de la Soc. d Biol.*, 1903, lv, 161; *J. de Phys. et de Path. gén.*, 1905, vii, 1.

Lewaschew.—*Arch. f. mik. Anat.*, 1886, xxvi, 452.

Lombroso.—*Arch. ital. de biol.*, 1904, xlii; *J. de Phys. et de Path. gén.*, 1905, vii, 3.

Lorand.—*Compt. rend. de la Soc. de Biol.*, 1904, lvi, 488.

Lüthje.—*Münchener med. Woch.*, 1903, l, 1537.

Mankowski.—*Arch. f. mik. Anat.*, 1901, lix, 286.

Minkowski.—*Arch. f. exper. Path. u. Phar.*, 1893, xxxi, 85.

Minot.—*Proc. Boston Society of Natural History*, 1900, xxix, 185.

Mouret.—*Compt. rend. de la Soc. de Biol.*, 1894, xlvi, 731.

Opie.—*Johns Hopkins Hosp. Bull.*, 1900, xi, 205; *Jour. of Boston Soc. of Med. Sciences*, 1900, iv, 251; *Jour. of Exper. Med.*, 1901, v, 397, 527.

Pearce.—*American Jour. of Anat.*, 1903, ii, 445; *American Medicine*, 1903, vi, 1020.

Pensa —*Internat. Monatsschr. f. Anat. u. Phys.*, 1905, xxii, 1.

Pflüger.—*Arch. f. d. ges. Phys.*, 1904, v, 106.

Pugnát.—*J. de l'anat. et de la physiol.*, 1897, xxxiii, 267.

Reitmann.—*Zeit. f. Heilkunde*, 1905, xxvi, 1.

Rennie.—*Quarterly Journ. of Microsc. Science.*, 1904, xlviii, 379; *Zent. f. Phys.*, 1905, xviii, 23.

Sauerbeck.—*Virchow's Archiv*, 1904, clxxvii, Suppl. Heft., 1; *Verhandl. der deutschen path. Gesellsch*, Ergänzungsheft; *Centb. f. path. Anat.*, 1904, xv, 217.

Schlesinger.—*Virchow's Archiv*, 1898, cliv, 501.

Schulze.—*Arch. f. mik. Anat.*, 1900, lvi, 491.

Šimácek.—*Zent. f. Phys.*, 1904, xvii, 3, 209.

Ssobolew.—*Virchow's Archiv*, 1902, clxviii, 91.

Stoklasa.—*Zent. f. Phys.*, 1903, xvi, 652.

Von Ebner.—*Kölliker's Handbuch der Gewebelehre des Menschen*, 1899.

Weichselbaum und Stangl.—*Wiener klin. Woch.*, 1901, xiv, 968.

Weintraud.—*Arch. f. exper. Path. u. Phar.*, 1894, xxxiv, 303.

Wright and Joslin.—*Jour. of Med. Research*, 1901, vi, 360.

Zunz.—*Zent. f. allg. Path. u. path. Anat.*, 1905, xvi, 5.