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EVIDENCE OF REGENERATION OF PANCREAS IN AN INSULIN TREATED CASE OF DIABETES *

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The peculiar pathology of the pancreas renders a knowledge of its histology essential to the understanding of any disease process involving it. The absence of any gross changes in the pancreas in many cases of diabetes has led to much confusion respecting the pathology of this disease and to some doubt of its being of pancreatic origin. Current literature tends to the view that changes are present when a close study of the histopathology of the organ is made.

Early in embryonic life three buds from the primitive intestinal tract develop, two ventral (Santorini) and a dorsal one (Wirsung). By the end of the second month of fetal life these anlagen fuse and give rise to all the structures of the adult pancreas. Pearce [1] and Küster [2] note separation and vascularity of cell groups in the third month. Such groups consist of four more or less distinct types of tissue — the ducts, centro-acinar cells, islet cells, and acinar cells. These structures are arranged in lobes separated by small amounts of connective tissue and again subdivided into lobules by the ramifications of the ducts. A typical lobule consists of a semi-spherical mass of cells into the centre of which a duct enters. In close proximity to the duct and possibly arising from it are numbers of flattened clear cells, the centro-acinar cells. The remainder of the lobule consists of pyramid-shaped acinar cells whose apices are

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filled with zymogenous granules, and the so-called islands of Langerhans. These were first described by Langerhans[3] in 1869 as small groups of cells studding the pancreas and differentiated from the acinar cells by their clarity and lack of zymogen granules. As early as 1886, Lewaschew[4] observed the presence of two types of cells in these islets differentiated by their shape and mode of delimitation. Ten years later Laguesse[5] described the presence of safranophile granules resembling zymogen granules but finer and more resistant to acetic acid. From this period until 1907 when Lane's work appeared, little progress was made in the study of the histopathology of the pancreas, the literature during this period being chiefly concerned with the origin of islets and a discussion as to their being a histological entity at all. The work of Lane[6] and Bensley,[7] through elaboration of technique and stains, has made possible a much clearer understanding of the composite cells of the islets and has shown that the early workers, because of their inability to differentiate clearly the acinar and islet cells, had given a wrong interpretation of their nature. These workers showed the presence of two, and possibly three, types of cells in an islet, the so-called A and B cells, and a third less differentiated Gamma cell. The cells of the islet are typically arranged in cords, but in many instances solitary islet cells are noted among a group of acinar cells. Some of these it is claimed are the result of artifacts. Islets are frequently found in close association with ducts but never actually communicate with them. This fact and their great vascularity led to the belief that these cells produced an internal secretion.

The frequent lack of gross pathological change in the pancreas has been a stumbling block to the acceptance of the theory of the pancreatic origin of the disease. The so-called simple atrophy of the pancreas with diminution in the number of islets alone, and accompanied in older patients by interacinar fibrosis, has been reported often. Pleasants[8] in 1900 described six cases of this type in children, considering it an evidence of congenital lack of development of these structures. A little later Ssobolew[9] reported fifteen such cases, in four of which no islets at all were observed. Failure of these observers to make serial sections and the lack of suitable staining technique may explain many of these negative findings. Hydropic degeneration of the islet cells, now regarded as pathognomonic of diabetes, was first described by Weichselbaum and

Stangl [10] in 1911, and shortly after, Homans [11] showed that these changes were confined to the B cells. Allen [12] has recently made elaborate studies of this type of degeneration and the factors influencing its development. He found definite vacuolation of the cells within five days of partial pancreatectomy. The remaining islet cells rapidly degnerate after this operation and in six weeks to two months the B cells are completely destroyed and replaced by acinar tissue with small groups of A cells interspersed. While under these conditions, the majority of vacuolated cells die; this may be prevented by the avoidance of over-function by means of diet and may allow recovery to occur. Such islands always remain small and may readily be overlooked without the use of suitable stains, giving the appearance of simple atrophy with decrease in the number of islets. Allen has also shown that active diabetes is a prerequisite to the development of these changes.

The following case was found suitable for reporting because of the clinical response to insulin associated with anatomical changes strongly suggestive of regeneration in the pancreas.

Clinical History: B. N. white male — age 9.

Family History: Father and one maternal uncle both have diabetes.

Diabetes was diagnosed in this child when he was two years old. He was placed on a suitable Allen diet, which was strictly adhered to, and for a time did well except for recurrent attacks of dysentery, which lowered his tolerance. Failure to gain in stature or weight in any way commensurable with his age was noted and the general conditon became worse each year until he was more or less a chronic invalid with increasingly frequent attacks of acidosis during the last year before starting insulin.

He was admitted to the Hospital for Sick Children, Toronto, the end of December 1922. At this time he was an emaciated dwarf, more or less drowsy and unhappy. His weight was 30 pounds and his height 39 inches. His tolerance to carbohydrate had decreased until he was now unable to utilize 15 grams of such food. Insulin treatment was started at once and his diet increased to a diet suitable for a boy of his age, sufficient insulin being given to keep him sugar free and his blood sugar normal. He was discharged on an adequate diet plus insulin. Progress both in general condition and in improvement of pancreatic function was steady.

The accompanying chart shows his decreasing need of insulin with his gain in weight. His tolerance to carbohydrate trebled in the year as shown either by the fact that 30 units of insulin controlled the disease as adequately as 90 a year before, or, stated in another way, without insulin he could now handle 45 grams of carbohydrate in place of 15.

The photographs taken when insulin was started and six months later indicated in some measure the improvement in general condition. From a chronic invalid in 1922 he became the leader of the "gang" in 1923. He was killed by

fracturing his skull when sleigh-riding. He lived only about three hours after receipt of the injury and an immediate post mortem examination was made.

Autopsy Findings: The pancreas, which was removed within thirty minutes of death, was immediately cut into small blocks, labeled and portions from the head, body, and tail placed in a 10-per-cent solution of neutral formalin and also in Zenker's fluid with acetic acid. The blocks were embedded in paraffin and sections from 2–6 microns were cut.

The formalin-fixed sections were stained in Ehrlich's hematoxylin and eosin. Some formalin-fixed material was further fixed in Bensley's acetic-osmic-bichromate mixture as for fresh tissue and later stained with Altmann's anilin acid fuchsin and methyl green. Cresyl violet was also used with some of this latter material to bring out nuclear contents more clearly.

The Zenker-fixed preparations were stained with a variety of special stains to show up their specific granules. The stains used were Bensley's neutral gentian violet, Martin's azo-fuchsin and basic ethyl violet, Bensley's safranin-acid violet, Altmann's acid fuchsin and methyl green, Mallory's phosphotungstic acid hematoxylin, and finally a stain by Bowie, to whom we are indebted for the privilege of using the method before publication, namely Biebrich scarlet and basic ethyl violet. This latter stain we found very satisfactory as a differential stain showing up as it does, with the acid Zenker's fixative, the granules of the A cells blue and those of the B cells red.

The pancreas in the gross, as in so many cases of diabetes, presented nothing abnormal. It weighed twenty-nine grams, which, considering the age of the patient and his condition, was good weight. Its color, shape, lobulation, and consistency appeared normal. On gross section nothing indicating a fibrosis, adipose replacement, fat necrosis, or hemorrhagic necrosis could be detected.

On histological examination of sections from different areas we were unable to find any distinctive lesions such as hydropic degeneration of islet cells, fibrosis or atrophy of the islets as are commonly described associated with diabetes. Neither could we detect associated lesions such as inter- and intra-lobular fibrosis, chronic pancreatitis, simple atrophy, or adipose replacement. The number and size of the islets gave no indication of the existence of a possible diabetes. The only finding was the presence of occasional pyknotic

nuclei in some of the islet cells. The negative findings just mentioned, however, are not inconsistent with diabetes. This has been pointed out by many observers and serves to prove that functional capacity of the islet cells, as determined by clinical laboratory tests, is after all the final criterion for determining the ability of the individual to utilize carbohydrates. With our present facilities we are not able histologically to estimate function and we must therefore rely upon clinical tests for this determination. While clinically, in this case, there was a marked rise in sugar tolerance, it never returned to normal and the case therefore remained one of diabetes. The rise in sugar tolerance would indicate either that the islets were not irreparably injured, or if so, that regeneration had taken place in others to compensate for the loss.

While nothing in the nature of a retrograde process was found in this pancreas beyond the presence of a few pyknotic nuclei in some of the islet cells, one could recognize many evidences of regeneration of the acinar, centro-acinar, and islet cells. About the margin of the pancreas the lobular arrangement became quite apparent and, in fact, was exaggerated into papillomatous structures composed of a garland of acinar cells surrounding a group of centro-acinar cells and drained by one of the radicles of the pancreatic duct. In some of the larger lobules there were small groups or cords of islet cells associated with the duct or centro-acinar cells. Throughout, there appeared to be a definite increase in the number of centro-acinar cells. These were readily identified with Bowie's stain by their large, oval-shaped-pinkish staining nuclei, with single or double nucleoli, and very little if any chromatin material and clear cytoplasm. This hyperplasia of the centro-acinar cells was most apparent in the peripheral portions of the pancreas.

Our attention was then directed towards a possible anatomical explanation of the increased functional capacity of the islet system.

Hypertrophy of the islets has been observed under various conditions and has been credited with the prevention of glycosuria in such cases. Opie [13] questions the functional efficiency of these islets, as similar islets are frequently noted in association with diabetes, probably in response to functional overstrain. Such hypertrophy has been reported in diabetes by Cecil,[14] and unassociated with glycosuria in cirrhosis of the liver by Ohlmacher,[15] and about the advancing margin of malignant growths by Pearce.[16] McCallum [17]

reported two cases of diabetes in children in whom there were large islets not sharply outlined because of apparent continuation of the cell strands with the acini. The acini were also hypertrophied. Weichselbaum [18] frequently observed such changes and considered them as regeneration by the outgrowth of solid columns of cells from the ducts. It would be impossible to be certain of the nature of the cells in these large islets with the technique used by these authors.

By the use of special islet cell stains described above, the islets stood out clearly and gave one the impression of a distinct increase in number over those observed with the ordinary stains. This increase can be explained by the presence of a number of islets made up of cells with large nuclei and having a very intimate association with the acinar cells, which with the ordinary strains were very difficult to identify. With the special stains, however, more particularly Bowie's stain, the islet cells were easily identified and the characteristic staining of their granules noted.

The size, distribution, and number of islets varied considerably in different areas of the same section. Some were quite large, others small, often consisting merely of a cluster or cord of six or eight cells. As far as we could tell with the number of sections studied, the distribution of the islets throughout the whole pancreas was fairly uniform, with possibly a few more islets in the tail. What was more striking was the vast number of islets to be found in certain peripheral areas of the cross section of the pancreas as compared with the more central portions. In some peripheral areas one could identify as many as twelve to fourteen islets in a low-powered field of the microscope, whereas in the central portions we found only one islet in two and three low-powered fields. It is of course not unusual in a fibrosed pancreas, or in one showing adipose replacement, to find the islets closely packed together. In this case, however, both of these factors were absent.

We were also able to identify certain differences in the islets of the peripheral portions from those of the more central areas which lead us to believe that, broadly speaking, we were dealing with two types of islets. This distinction was not made upon characteristics of the individual cells, but rather upon their distribution, size, shape, structure, and percentage of A cells to B cells present. Neither is it suggested that they might be functionally different beyond the possible effect of a deficiency of A cells in the one group.

Throughout the central portions of the pancreas the islets as a rule were larger and contained more islet cells than one normally finds. Their cells were closely packed together and many of them showed pyknotic changes in their nuclei. They lacked the orderly structural arrangement in cords, and appeared jumbled. The outline of the islet was very irregular and islet cells could be seen extending from the main group into the surrounding acinar tissue for varying distances. No capsule, therefore, could be distinguished, although there appeared to be a normal amount of stroma supporting the individual cells. The ratio of A cells to B cells was approximately normal, the A cells representing from twenty to fifty per cent of the total number.

In the peripheral portions of the pancreas and in the centers of the finer pancreatic lobules we found islets having certain morphological changes which set them apart from those just described. As a rule they were not so large as those first described, often consisting of not more than six or eight cells. While they had no apparent capsule, they were well defined by the pronounced staining characteristics of their cells, which were also arranged in definite cords or well-defined round masses. Their cells appeared larger than the cells of the first type of islet. A small amount of connective tissue stroma supported these cells and in it were one or more blood capillaries. Their close association with the finer duct radicles and with groups of centro-acinar cells was quite obvious. In some cases they could be distinguished from centro-acinar and duct cells only by the specific staining of their granules. As an evidence of the more active growth of the islet cells we very often found them compressing the surrounding acinar cells into thin, irregular, deeply staining masses of cytoplasm. The most striking feature of these islets possibly was the almost entire absence of A cells. Occasionally we could find one or two A cells, but for the most part they were composed entirely of B cells, which were quite large with large, round or oval reticulated nuclei and which showed in their cytoplasm, with Bowie's stain, large numbers of reddish granules.

From these findings we were convinced that here we were dealing with islets which were possibly in age or time of origin different. The large central islets showing evidences of pyknotic degeneration and the lack of an orderly arrangement of their cells, and numerous A cells, we felt were older islets which existed prior to the insulin

treatment. Their increased cellularity and the spreading out of their cells into the surrounding acinar tissue suggested some attempt at regeneration.

The very numerous islets found in the peripheral areas we believe represent younger islets. This conclusion is based upon the larger size of their cells as compared with the former, their orderly arrangement in cords, absence of pyknosis, their very close association with groups of centro-acinar cells, and by the presence of practically the one type of cell, the B cell.

In view of the fact, as pointed out by Allen [12] and Homans [11] that the B cells are the antidiabetogenic cells and the ones first destroyed in severe diabetes, it is interesting to note in this case the almost complete dominance of B cells in so many of the peripheral islets as compared with those of a normal pancreas or of the central areas of this pancreas. These islets we believe are new islets which have probably developed since the commencement of insulin treatment and in functioning have been responsible in a measure for the rise in sugar tolerance. Their distribution and prominence in the peripheral portions of the pancreas suggest very strongly that they are new islets forming in the natural growth of the pancreas. The insulin treatment in relieving the strain placed upon the already functionally deficient islet system allowed the new islets to develop and assume their normal functional capacity.

Discussion by Prof. R. R. Bensley

I am very happy to be here today and to hear this case presented by Dr. Gladys Boyd. There is perhaps no problem so difficult for the pathologist to approach as that of explaining, on the basis of histological changes, why the patient improves. This case of pancreatic response to insulin treatment gives us new hopes, and increases the responsibility of the pathologist in the investigation of similar material. The interpretation of such changes as Dr. Boyd has pointed out to us is very difficult. In the absence of controls one can only imagine what the condition of the pancreas was prior to the insulin treatment, and attempt to read the process on the basis of this hypothetical condition. In Dr. Boyd's case I was more impressed with certain groups of small bulbous islands, clustered round a duct, which strongly resembled in morphology and arrangement the regenerative islands seen in experimental animals, than by

the other evidences of regeneration which she has enumerated. I have little doubt that these were actually new island units. In these cases the application of the vital-staining methods would undoubtedly render great service. I realize the difficulty of applying such methods to the study of the human pancreas, but it was accomplished successfully by Clark in several cases. The advantage of these methods is that they permit the intelligent inspection of the whole pancreas. It is the lack of such comprehensive study that is responsible for much of the confusion in interpreting the gland.

The literature of pancreatic regeneration is most unsatisfactory. A good deal is quite unreliable because of the difficulty of distinguishing between regressive and regenerative changes. The changes described by Kyrle are to my mind largely regressive. De la Roche, working under the direction of Laguesse, studied the events which followed the ligation of the pancreatic duct and found that there was considerable effort to reconstruct both acini and ducts. Some years ago I presented to the Harvey Society a description of some findings of Clark encountered in the study of similar cases of duct ligation. He found that, contrary to the usual opinion, acinous tissue did not disappear wholly until a long time after duct ligation, though the destruction of this tissue was extensive. It is fortunate that Banting supposed that the acinous tissue did disappear under these conditions, since we owe to this idea the discovery of insulin. What Banting had was probably a pancreas still containing abundant island tissue but reduced to its lowest ebb of zymogenic activity. After ligation of the duct the process was wholly destructive for a time, involving both islands and acinous tissue, but the latter more than the former. At the end of a month regenerative processes became dominant, again involving both tissues, and resulting in the formation of new acini and new islands. The newly formed acini, since they have no outlet by the duct, in turn succumb to the destructive process, but the new islands increase progressively until a maximum is reached.

In this series of experimental animals a few were encountered in which autopsy, several months after ligation of the duct, revealed a complete pancreas differing from the normal only in the fact that it was smaller in size than normal, and contained more fibrous interstitial tissue. Since this change was entirely confined to the pancreas, we interpreted these as cases in which a successful ligation

of the duct had been followed by the usual regressive changes, but in which the accidental reëstablishment of the communication of the duct with the bowel had permitted a complete regeneration of the pancreas. In one of these cases the duct had found a new opening at some distance from the stump of the old duct, which was identified at autopsy. This interpretation has been confirmed recently in my laboratory by my student Grauer, who undertook the task of reimplanting the duct into the bowel a month after a successful ligation of the duct. This reimplantation presents great difficulties and so far he had had only two successful cases out of about forty-five attempts. In every case controls of the pancreas are taken at the time of reimplantation. In one of these cases he found one month after reimplantation a complete pancreas similar in all respects to those observed by Clark. The control tissue from this animal taken at the time of reimplantation showed a typical one-month ligation picture. In the second case, examined two weeks after reimplantation of the duct, the acini were growing out into the fibrous envelope of the pancreas and covering the newly formed islets. On the basis of these experiments and observations it is my opinion that, given appropriate conditions, the capacity of the pancreas for regeneration is one hundred per cent. I believe that we shall ultimately learn how to control it. It is significant that in these observations the regenerative emphasis seems to be on the island tissue as long as the duct remains closed, but shifts to the acinous tissue as soon as communication with the bowel is reëstablished.

It is easy to see from these experiments that a pancreas obtained at autopsy in a case of diabetes may have passed through a series of kaleidoscopic changes which it would tax the imagination to figure out. For this reason great caution is necessary in the interpretation of such material.

The greatest dilemma of all in applying the insular hypothesis to the pathology of diabetes is presented by those cases in which no changes can be demonstrated in the pancreas. Various attempts have been made to explain these cases on a basis of numerical reduction of the islands, on reduction in size or in functional competency. I regret to say that I have little confidence in the results of enumeration of islands in sections. The amount of tissue which can be inspected by such methods is too infinitesimal to warrant

general conclusions. It must be remembered that we know very little about the mechanism of physiological control in the endocrine organs, and until we know more it is futile to speculate. It would be better to attempt no explanation at all of these difficult cases than to yield to the temptation to adapt the facts to the insular hypothesis. Meanwhile, such cases as the one presented by Dr. Boyd are of great importance, and will, no doubt, reward careful study by the pathologist.

Reply — Dr. Boyd

I would just like to read to you extracts from letters from Dr. F. M. Allen and Dr. Eugene Opie, giving their opinion on this case.

1. Dr. Allen: "I have the impression, first, that the islands are more numerous than should be anticipated in a child with the low tolerance which you mention before insulin treatment. Furthermore, some of the islands are large and typical, but small and irregular islands are more common than normal. They have the true capillary and trabecular framework, but the capsule is often not visible and they merge with the surrounding acinar tissue with appearances strongly suggesting 'transitions.' The special granule stain as usual excludes any true transitions between island and acinar tissue, and shows furthermore that the cytology of the islands is normal. It is altogether probable that some children at least should have the power of forming new island tissue. It has been rather surprising that this process, which must be a regular occurrence in the growing pancreas of the normal child, has been so deficient in diabetic children under diet treatment. From the combined clinical and microscopic evidence, I think you have grounds for the belief that new formation of island tissue has occurred in this case under insulin treatment. Presumably this growth has occurred by enlargement of existing islands or prolifition of ducts."

2. Dr. Opie: "I have received the second set of slides from your case of diabetic pancreas. I find in them very definite evidence of hypertrophy of the islands of Langerhans, with columns of cells extending into the acinar tissue."

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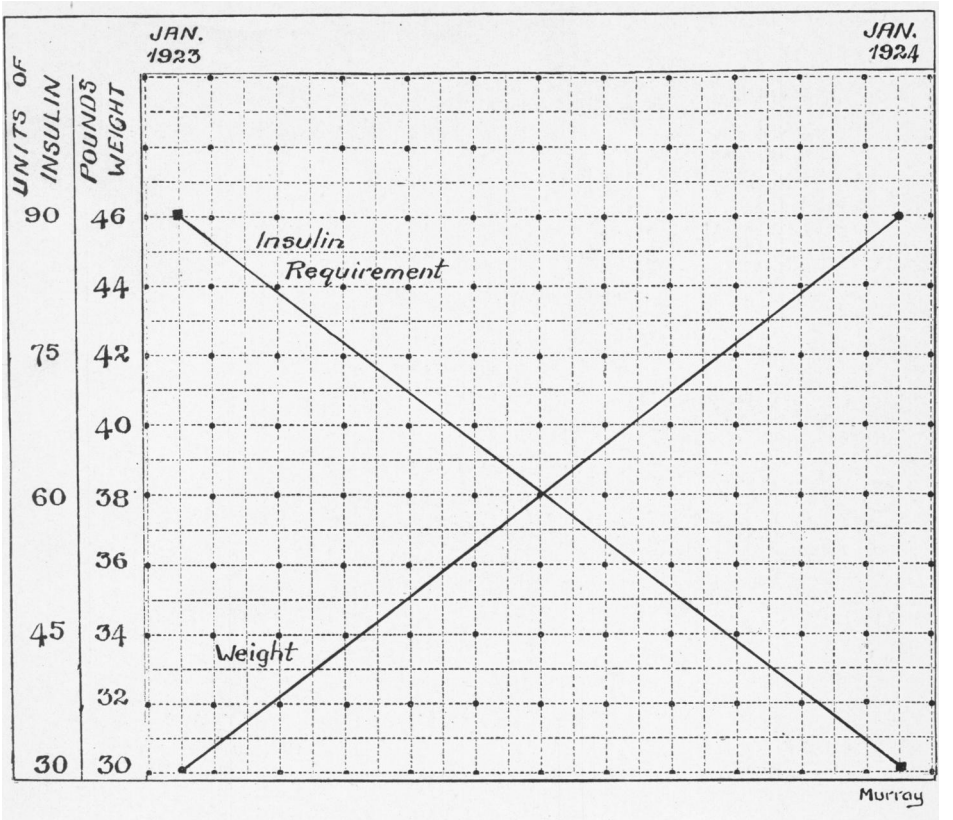
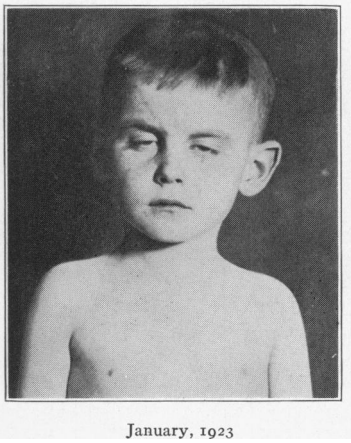
DESCRIPTION OF PLATE XXII

Chart showing decrease in insulin requirement and gain in weight of case reported.

Photographs of B. N. in January and in June, 1923.





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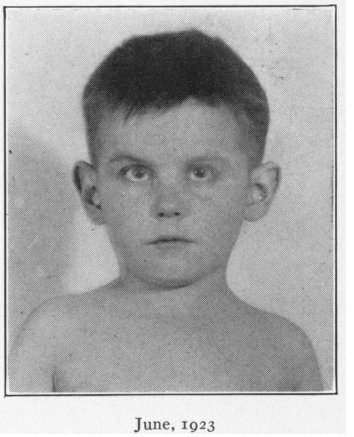

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