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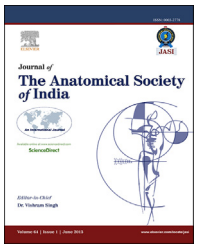

Original Article

Prenatal development of the human endocrine pancreas: A morphological and immunohistochemical study

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ABSTRACT

Introduction: The endocrine pancreas plays a pivotal role in glucose metabolism. As regards the morphogenesis of the islets of Langerhans, there is conflicting data regarding the timing of appearance of the B cells, and, the proportion and arrangement of the B cells within the islets. The present work is a baseline study conducted in the Indian subcontinent. The histogenesis of the islets of Langerhans was studied and we also observed the expression of anti-insulin antibody in the islets at different gestational ages.

Methods: Ten aborted fetal specimens of pancreas of gestational ages 10–36 weeks were procured from the Department of Obstetrics and Gynaecology, LNJP Hospital, New Delhi. Fetuses were fixed in 10% formalin. Serial sections were stained with Haematoxylin and Eosin and few sections were processed for immunocytochemistry with a specific marker for B-cell, the anti-insulin antibody.

Results: The cells of the islets arise from the lining epithelium of the tubules. The B cells contain insulin at 10th week as seen by immunostaining. Small capillaries are seen enclosed in the islets at 14 weeks. The arrangement of B cells in different islets is variable. The formation of islets continues throughout fetal life.

Discussion: Our study reaffirms that the endocrine pancreas begins to differentiate early in fetal life. The growth and maturation of islets is associated with coordinated vascular development. By the 28th week of intrauterine life, the fetal pancreas attains sufficient morphological maturity so as to fulfil the hormonal requirements of the growing fetus.

1. Introduction

The islets of Langerhans, the endocrine component of the pancreas, are complex micro-organs involved in glucose homeostasis. Seven different types of cells have been found in the islets – A, B, D, F, D_1, EC (enterochromaffin cells), G1 (gastrin) cells. The B cells are the most common and account for 60–75% of the cells in the islets.[1] B cells mainly secrete

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insulin and islet amyloid polypeptide (IAPP).[2] Insulin plays a key role in glucose metabolism. Deficiency of insulin results in diabetes mellitus. Research has shown that pathological changes occur in the pancreas especially in the islets in diabetes mellitus. Diabetes mellitus is the most common metabolic disorder affecting millions worldwide. India has the highest number of estimated cases of diabetes in the world.[3] Type 1 diabetes or Insulin Dependent diabetes patients need a regular external replenishment of insulin in order to lead a normal life. Diabetes can also occur as early as first six months of life known as monogenic diabetes. Research shows that in diabetics, pathological changes occur in the pancreas especially in the islets.[4,5] Thus, the embryogenesis and renewal of the islets is of critical importance in diabetology. A detailed knowledge of the normal development of the islets will help in understanding any developmental anomaly that may trigger the pathogenesis of diabetes. There are many such developmental studies in lower mammals and invertebrates. But, there are wide interspecies differences between the islets of humans and other mammals.[6,7] Hence, data from studies on lower mammals cannot be extrapolated onto humans. This also explains why treatment modalities for diabetes which were found successful in rodents did not produce similar results in humans. Factors that affect islet cell development in the higher mammals, especially human pancreas are not well known. There are some human studies but they show variation in the data regarding the time of appearance of hormone containing B cells. Hence, we have taken up this study to understand the embryogenesis of pancreas, especially the endocrine component, in humans in the Indian subcontinent. It is a baseline study of the sequential development of the parenchyma with particular focus on the histogenesis of the islet cells.

2. Materials and methods

Fetuses of gestational age 10–36 weeks were procured from the Department of Obstetrics and Gynaecology, L.N.J.P. Hospital, New Delhi after obtaining approval from institutional ethical committee of Maulana Azad Medical College and associated L.N.J.P. Hospital. Informed consent of parents was taken and patient anonymity was preserved. Fetuses below the gestational age of 20 weeks were obtained from abortions conducted in accordance with the Medical Termination of Pregnancy act, while those above 20 weeks of gestation were obtained from stillbirths. A detailed maternal history was recorded and diabetic mothers were excluded from the study. Patient anonymity was preserved. An initial assessment of the fetus was done to rule out any gross abnormality. Only normal fetuses were included in the study. The gestational ages of the procured fetuses was determined by measuring- Crown-Rump length, Crown-Heel length, Bi-parietal diameter and Foot Length.

Incision was given longitudinally on the anterior abdominal wall in the median plane for better penetration of the fixative into the abdomen. The fetus was then immersed in 10% paraformaldehyde. After fixation, the pancreas was dissected out and preserved in fresh fixative for 1–2 weeks. The specimens were labelled and processed for paraffin embedding. 7 μm thick serial sections were generated on a rotary microtome with the long axis of pancreas as the cutting surface.

3. Staining

Sections were stained with haematoxylin and eosin (H&E) stain to see the morphology of the developing pancreas. In each fetal pancreas, few sections were processed for immunohistochemistry (IHC). Deparaffinised sections were incubated in citrate buffer and the endogenous peroxidase activity was blocked using methanol and 1% H_2O_2. After washing with working solution of phosphate buffer with 0.1% Triton X, slides were treated with normal horse serum for 2 h for blocking the non specific antigen. The sections were then incubated with monoclonal anti-insulin antibody at a dilution of 1:200, at 4 °C overnight. Slides were then treated with biotinylated secondary antibody, and the reaction was observed using diaminobenzidene as chromagen.

All stained sections were assessed qualitatively under the BX 61 computerised microscope and the images were captured with Olympus DP71 camera. Processing of images was done using the Image Pro plus MC 6 software. The B cells, stained for anti-insulin antibody were observed and analysed.

4. Results

10 weeks: At this gestational age, the dorsal and ventral pancreatic buds had already fused to form one single mass. A thin connective tissue capsule was seen around the gland. The parenchyma of the gland at this stage consisted of large amounts of mesenchymal tissue with an interspersed network of branching tubules. The tubules were lined by columnar epithelium with cells having a lightly eosinophilic cytoplasm and oval vesicular nuclei present towards the base. The nuclei had a prominent nucleolus. It was difficult to distinguish between the cells in the terminal tubules and the primitive acinar cells (Fig. 1a). Immunostaining revealed that few cells in the lining of tubules showed a positive reaction with anti-insulin antibody, thus confirming the presence of B cells in the lining of the tubules (Fig. 1b).

14 weeks: By the 14th week, the parenchymal tissue had started organising into vaguely defined lobes. The parenchyma contained branching tubular ducts lined by columnar to stratified epithelium. Cells were budding out from the tubules especially from the stratified areas in the form of cords and small clusters. Many islets were in the stage of budding out from the tubules. The developing islets were enclosing more and more capillaries within them. Some islets had detached from the tubules and were seen in close proximity to the tubules. These were mostly small islets with few scattered loosely packed cells (Fig. 2a). Immunostaining showed many more insulin-positive B cells in each section at this age. The cells were more intensely stained than in younger fetuses. There were single B cells present in the lining of tubules. B cells were also seen in the budding out islets. Very few large islets were seen in the tail region of the pancreas (Fig. 2b). In

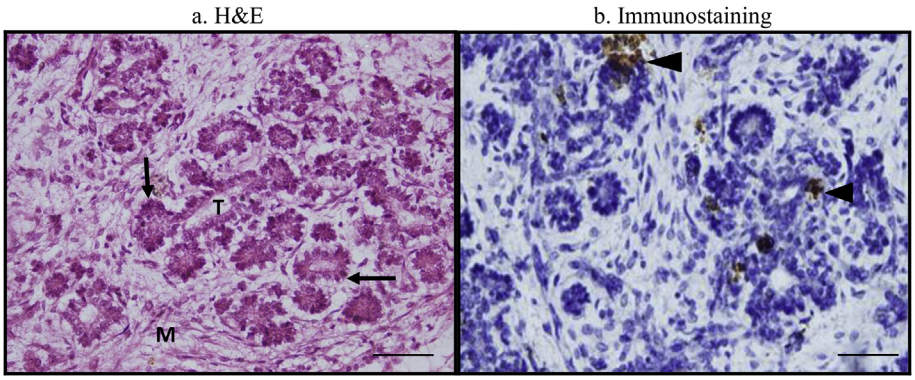

Fig. 1 – 10 weeks: a) Haematoxylin & Eosin (H&E) staining showing parenchyma of the developing pancreas containing abundant mesenchymal tissue (M). The branching tubules (T) lined by columnar epithelium (arrowhead) are embedded in it. Scale Bar: 1 cm = 15 μm. b) Immunostaining in the pancreas demonstrates B cells showing a positive reaction with anti-insulin antibody (brown colour). These are located mainly in the walls of tubules (arrow) and in very small groups budding out from tubules (arrowhead). Scale Bar: 1 cm = 15 μm.

the head region, majority of the islets were small and in the stage of budding out from the ducts.

18 weeks: From the 18th week onwards, the connective tissue capsule around the gland was well defined. There were distinct septa in between the lobes. The ducts were more frequent than the tubules and were lined by columnar epithelium. The acinar cells showed faint apical acidophilia and basal basophilia. The cells in the islets were stained lighter than the acini in the H&E stain. The cells were polyhedral and loosely packed in contrast to the compact arrangement in the acini. Their nuclei were rounded and well defined. The islets were usually in close association with the ducts. These were well encapsulated by a delicate connective tissue capsule. They were now associated with multiple small blood capillaries. IHC revealed that majority of the B cells were mostly concentrated in the core of the islets.

24 weeks: By the 24th week, the anlage of pancreas had increased in both length and breadth. The parenchyma had proliferating acini and some large ducts lined by low columnar epithelium. The islets were seen in various stages of development. Their sizes vary from small clusters to large islets. The islet showed a rich blood supply with many small arterioles and capillaries (Fig. 3a). On performing IHC, some islets showed a peculiar arrangement of B cells. Most of the B cells in these islets were clustered at one pole. The other endocrine cells were at the other pole (Fig. 3b). Also, in many islets, proportion of B cells was almost equal to the other endocrine cells or even less. The islets were larger and more numerous compared to the younger fetuses, especially in the tail and body of the pancreas.

36 weeks: By 36 weeks of gestation, the architecture of the lobes and lobules was very well developed. The islets were easily recognisable. Most of the islets were very large. Their cytoarchitecture was well developed with a well established microvasculature. They were pale staining compared to the acini. The islet cells had a prominent nucleus. The cytoplasm

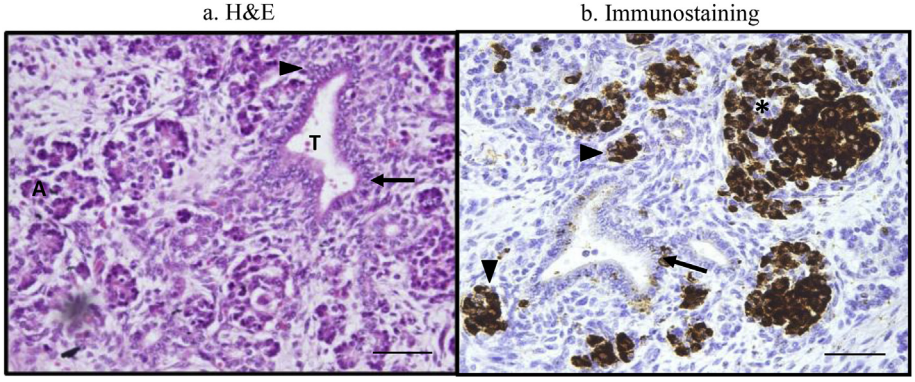

Fig. 2 – 14 weeks: a) H&E staining depicting the vague organisation of acini into lobes (A). Many branching tubules (T) with a wide lumen are seen. They are lined by columnar epithelium. The cells show a light eosinophilic cytoplasm, well defined, elongated nuclei present towards the base (arrow). At places, the tubules show stratified lining epithelium (arrowhead). Cells are seen budding out from the tubules especially from these areas in the form of cords and small clusters. Scale Bar: 1 cm = 15 μm. b) Immunostaining for anti-insulin antibody shows brown stained B cells in the islets. The islets are in various stages of development ranging from: single B cells present in the lining of tubules (arrow) to small clusters seen budding out of the tubules (arrowhead). Few islets have attained a large size (*). Scale Bar: 1 cm = 15 μm.

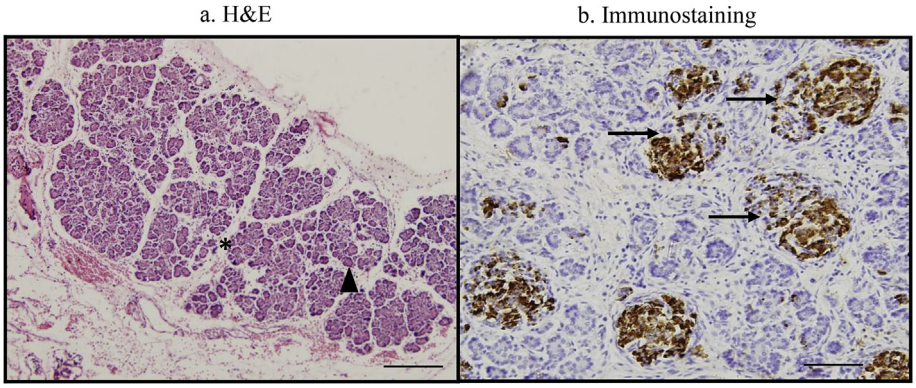

Fig. 3 – 24 weeks: a) H&E staining showing differentiation of the pancreas into lobes separated by well defined connective tissue septa (*). The acinar cells showed a basal basophilia and apical acidophilia (arrowhead). Scale Bar: 1 cm = 100 μm. b) Immunostaining showing the tail region of pancreas with many large islets. The arrangement of the islets is "Bipolar" with most B cells concentrated at one pole of the islet (arrow). Scale Bar: 1 cm = 15 μm.

was granular (Fig. 4a). At this age, when the IHC sections in the region of head, body and tail were compared, it was found that all regions showed islets in various stages of development. But, the size of islets was largest in the tail region (Fig. 4b). The head region showed small islets many of which were in the stage of budding out from the ducts. Some B cells could still be seen in the lining of the ducts (Fig. 5a). Large islets were scant in the head region. The cell composition varied from islet to islet. But, by large, the B cells stained with anti-insulin antibody were the most numerous cells in most of the islets. In some islets, B cells were not in majority (Fig. 5b). The arrangement of the cells was variable. Most of the islets however showed that the B cells were concentrated towards the core of the islets (Fig. 5c). Some islets showed B cells to be scattered uniformly throughout the islet (Fig. 5d).

5. Discussion

The study of the development of human endocrine pancreas is of critical importance in diabetology. The islets of Langerhans are of immense clinical importance because they are site of formation of the hormone, insulin, which becomes scarce in diabetes mellitus. The previous studies observe conflicting data with regards to the time of appearance of the endocrine cells, the arrangement of the cells within the islets, the proportion of the various cell types and the time of onset of secretory activity in the islets. The differences can be attributed to some extent to inadequate estimation of fetal age. Our study was thus directed towards understanding the development of human fetal pancreas with special focus on the histogenesis of the islets. We correlated findings from H&E stain with immunostaining using a specific marker for B cell, the anti-insulin antibody. Hence, it is a comprehensive study that elucidates the morphological maturation of the islets of Langerhans sequentially at different gestational ages.

The pancreas develops from two evaginations of the foregut endoderm– a dorsal and a ventral pancreatic bud, which later fuse to form a single organ. Thus the inferior part of the head of pancreas and the uncinate process are derived from the ventral bud while the rest of the head, neck, body and tail are derived from the dorsal bud.[8]

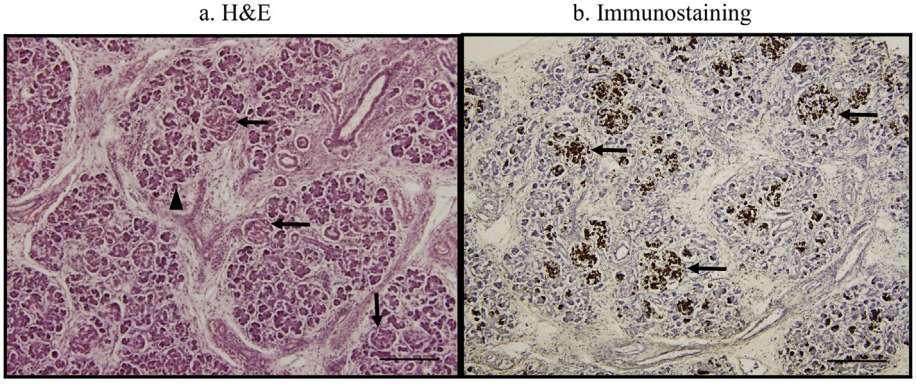

Fig. 4 – 36 weeks: a) H&E in the pancreas showing a distinct lobular architecture. The acini show well defined basal basophilia and apical acidophilia in the pyramidal acinar cells (arrowheads). Large islets are seen surrounded by a connective tissue capsule (arrow). Scale Bar: 1 cm = 100 μm. b) Immunostaining of the tail region of pancreas showing islets in various stages of development and many large islets (arrow). Scale Bar: 1 cm = 100 μm.

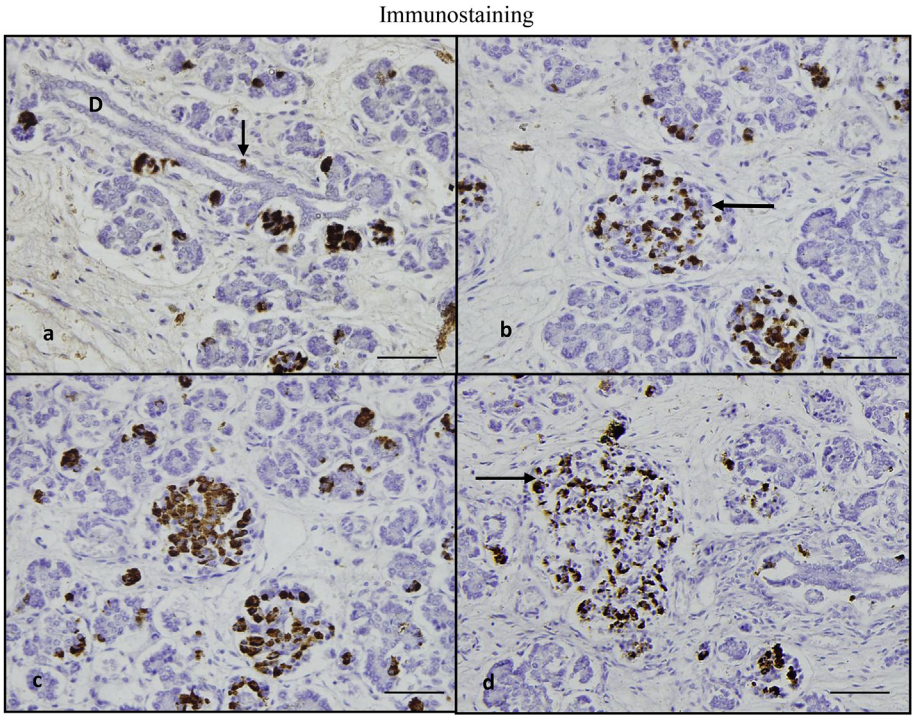

Fig. 5 – 36 weeks: a) Immunostaining showing the head region of pancreas with small islets containing fewer cells. A single B cell (arrow) showing positive reaction with anti-insulin antibody is seen in lining of the duct (D). Scale Bar: 1 cm = 15 μm. b) Immunostaining showing an islet containing very few B cells (arrow). Scale Bar: 1 cm = 15 μm. c) Immunostaining showing an islet containing B cells concentrated towards the core of the islet (arrow). Scale Bar: 1 cm = 15 μm. d) Immunostaining showing an islet containing B cells uniformly scattered throughout the islet (arrow). Scale Bar: 1 cm = 15 μm.

The youngest fetus in our study belonged to the 10th week of gestation. The pancreas could be easily differentiated as a glandular structure developing in the connective tissue around the duodenum. The gland had abundant primitive mesenchymal tissue in which were embedded branching tubules. Literature reveals that epithelio–mesenchymal interactions are very important in pancreatic organogenesis.[9] Immunostaining revealed distinct B cells in the duct walls or budding out from them. Hence, we can say that by 10th week of intra-uterine life, endocrine B cells had already begun differentiating from the duct walls. Our findings correspond to the study by Robb P who observed B cells at 10 weeks of gestation.[10] However, some studies report a higher age of appearance of B cells. Like and Orci found B cells to appear at 10.5 weeks and Falin states that B cells appear at 10–11 weeks.[11,12] Another study reports cells expressing insulin immunoreactivity as early as 52 days post conception.[13] At 10 weeks stage, we did not see the predominance of either B or other endocrine cells. However, Conklin stated that A cells were in a majority.[14] Others state that both A and B cell co-expression was found at around 8 weeks.[15] These divergent results may be due to difference in histochemical techniques and also inadequacy in the estimation of fetal ages.

In the early stages, most endocrine cells are either located in the walls of the tubules, or in close proximity to them as small primitive islets. This suggests that the epithelium lining of the tubules serves as the precursor for both islet cells and acinar cells.

In the 14 weeks old fetus, the parenchyma had less of primitive mesenchyme and branching tubules with a wide lumen were present. The islets were now beginning to enclose small capillaries indicating that the endocrine cells may be secreting hormones into the bloodstream. Our findings are concordant with other studies.[10,13] At this stage, the proportion of B cells is slightly higher than the other endocrine cells. This is in agreement with other works.[12] The B cells intermingle with the other endocrine cells.

As the gestational age advanced, the general lobular architecture of the pancreas was more mature. The islets were larger and more numerous than the younger fetuses. The larger islets were well encapsulated. Multiple blood capillaries are now associated with them signifying that functional maturation of islets is associated with coordinated vascular development. The importance of vascular supply for islet development has been highlighted by a series of studies in mice. It has been demonstrated that vascular endothelial cells provide signals for embryonic islet cell development.[16] Consistent with these studies we noticed islets to primarily develop alongside blood vessels and a dense capillary network was seen in the mature islets. Immunostaining revealed that even now, some B cells were present in the duct walls, thereby, indicating a continuous formation of new endocrine cells. The intensity of immunostain

was much more compared to younger age groups. The B cells are seen mostly in the central part of the islet. This corresponds to observations of other authors.[17]

By 24 weeks of gestational age, we found that the lobular architecture and the connective tissue in the stroma had become more evolved. The parenchyma had fewer tubules. This is because they have differentiated into ducts. The islets were seen in various stages of development, which were not always present close to ducts. This implies that the islets have matured and established themselves as independent entities. Some single cells were also seen in the duct walls. Multiple capillaries were seen in the islets. Most of the islets had a major proportion of B cells and most of the B cells were present in the centre of islets. The significance of the central grouping of B cells is not clear. In some islets, B cells were intermingling with other endocrine cells. The bipolar islets found at this stage have been reported by some authors at 16 weeks of intrauterine life and by others from 30 weeks onwards.[10,18]

In the 36 weeks old fetus, the pancreas had acquired a mature appearance. Islets were seen in various sizes and had a well-developed vascular system and delicate intra-islet connective tissue. Primitive tubules have almost disappeared. Very scant single B cells could be seen in immunostained sections, mainly in the walls of interlobular and intralobar ducts. The proportion of B cells and their distribution patterns within the islets were similar to studies by other authors.

Hence our study confirms that the endocrine pancreas begins to differentiate early in fetal life (before 10th week). The islets aggregate and undergo a phase of rapid growth and maturation. The presence of granules that take up the special stains and their intimate association with blood vessels indicate that they acquire functional maturity and are releasing the hormones into the bloodstream by 14 weeks of age. This is in contrast to the slower development of the exocrine acini where the shape and staining characteristic of cells undergo transformation at around 20 weeks. Islet cells are functional during fetal life and have a role in glucose metabolism of the fetus. This is proven by studies that show that as a result of hyperglycaemia in fetuses of diabetic mothers, islets undergo hypertrophy and hyperplasia.[19]

6. Conclusion

The formation of new islet cells from the ducts continues throughout fetal life. The adult islets show varying pattern of distribution of cells but as far as proportion of cells is concerned, B cells are the most common type in most of the islets. It is evident that by 28 weeks of age, the pancreas attains considerable morphological maturation thereby indicating that the fetal pancreas should also be functionally mature by this age in order to meet the increasing demands of the developing fetus. The understanding of the normal development of islets will help in identifying any abnormalities during development that might contribute to the pathogenesis of diabetes mellitus in the intra-uterine life.

Conflicts of interest

All authors have none to declare.

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