amorphous material may include assembly sites that are prepatterned on the rootlets. Such an arrangement would permit the control of patterning and directionality of microtubule assembly. At least some of this capacity is retained in vitro (Fig. 4a). The rootlets do not simply serve as attachment sites for assembled brain microtubules but, in fact, appear to promote tubule polymerization under conditions where little or no free microtubule assembly occurs.

The composition of the amorphous material associated with presumptive microtubule organizing centers in vivo (*3*) is not known. Weisenberg and Rosenfeld (*15*) have reported the isolation of an apparently unstructured aggregate from surf clam eggs which will serve as a microtubule organizing center for the in vitro assembly of microtubules into asters and spindles. It is possible that this aggregate and the amorphous material of other organizing centers may be localized high concentrations of tubulin subunits that can be assembled into microtubules. It seems equally possible that such structures contain other components which influence or are necessary for initiating tubule polymerization. The in vitro polymerization of brain tubulin (6*S*) subunits has been shown to require the presence of disks or rings (*16*) which consist of tubulin and additional protein components (*17*). It is not known if such ring structures are related to assembly of microtubules at specific sites in vivo. The procedures described in this report should permit an analysis of the role of rings and accessory proteins in an in vitro assembly system which, at least in some respects, resembles the in vivo situation.

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The Neural Crest and the Origin of the Insulin-Producing and Other Gastrointestinal Hormone-Producing Cells

Abstract. *It has been proposed that the endocrine cells of the digestive tract derive from the neuroectoderm (neural crest). To test this hypothesis we removed the entire ectoderm, the precursor of the neural crest, of embryonic rats prior to the formation of the neural crest and cultured the mesoendoderm for 11 days. In every case where a pancreas developed, insulin was detected or B cells were observed. Thus, a neural crest origin for these cells is eliminated.*

The neural crest is a temporary embryonic structure which appears along the neural fold concomitant with its closure to form the neural tube. Soon after the formation of the neural crest, the cells migrate to different parts of the embryo to give rise to a variety of otherwise unrelated differentiated cells. Some of these, like the chromaffin adrenomedullary cells and cells of the sympathetic ganglia, synthesize and accumulate high levels of amines as specific products. This peculiarity gives them the property of fluorescing under ultraviolet illumination after fixation with formaldehyde vapor (*1*). A similar reaction also occurs in some cells which can concentrate injected 3,4-dihydroxyphenylalanine (*2*). The cells which exhibit this fluorescence reaction have been termed by Pearse (*3*) APUD (amine precursor uptake and decarboxylation) cells.

Cells that have the histochemical specific characteristics mentioned above have been found in various embryonic and adult tissues, in particular, islet cells and gut epithelium (*4*). With the implicit assumptions that all cells which show the fluorescent histochemical reaction are derived from the neural crest and that the fluorescent cells in the gut are polypeptide hormone (for example, gastrin, glucagon, secretin) producing cells, Pearse proposed that all the polypeptide hormone–producing cells of the digestive tract originate from the neural crest and not from the endodermal cells as was previously thought (*5*). This intriguing theory, which would developmentally link both nervous and hormonal control to a common ectodermal origin, has been tested in only one case: Polak *et al.* (*6*) reported that the parafollicular or C cells which produce calcitonin, a polypeptidic hormone involved in calcium metabolism, are derived from the neural crest. By grafting the neural tube and crest of embryonic quail to chicken embryos, it is possible to follow the migration and fate of neural crest cells of the donor into the recipient tissues, because the nuclei of quail and chicken cells can be distinguished morphologically (*7*). This heterograft technique was combined with immunofluorescent labeling of calcitonin, and quail cells containing calcitonin were detected in the chicken thyroid. Thus, it was concluded that calcitonin-producing cells originate from the neural crest (*6*).

We have tested whether the pancreatic B cells (a typical APUD cell according to Pearse) originate from neural crest cells by culturing the developing embryo in vitro after removal of the ectodermal precursor of the neural crest. If the endocrine B cells were in fact derived from the neural crest, then they should be absent from the pancreas of these ectoderm-deprived embryos. For these experiments, whole 9-day rat embryos prior to four somites (Fig. 1) were removed aseptically from the mothers. At this time the neural groove has not closed to become the neural tube, and the neural crest has not yet formed. After resection of the extraembryonic area, the embryos were incubated in 3 percent trypsin-pancreatin for about 10 to 15 minutes, after which the whole ectodermal sheet was peeled off to completely remove the source of the neural crest cells. Figure 2 demonstrates the effectiveness of this procedure. A cross section of the mesoendoderm (not shown) confirms the absence of the ectodermal component. After several washes in serum to remove the trypsin, the mesoendoderm (Fig. 3) was cultured with Eagle's basal medium in which the amino acids were increased six-

fold. This medium was supplemented with 10 percent embryo extract and 10 percent mesenchymal factor in order to optimize growth and development (*8*).

The tissues were cultured for 11 days, the time it takes for the pancreatic cells to differentiate in vivo. In about a quarter of the cases, a pancreas was clearly visible with the dissecting microscope. The failure of the other cultures to develop a pancreas is probably not a result of the removal of the ectoderm, since, as previously reported (*9*), only a minority of the complete (with ectoderm) early embryonic explants develop a pancreas in vitro. The cultures were either harvested for insulin assay or fixed for morphological observations. Insulin and B cells in approximately normal proportions were found in every culture in which an exocrine pancreas was present. Figure 4 shows a part of a typical B cell found in these ectoderm-deprived cultures. When rudiments explanted at three somites or less were cultured, the amount of insulin found in the differentiated pancreas was between 0.5 and 2 ng of insulin per pancreas. If it is assumed that the B cells in the cultured rudiments contain ~ 10 pg of insulin, the amount present in the mature B cells (*10*), then the insulin content corresponds to ~ 50 to 200 cells. This approximates the normal proportion of B cells expected in a pancreas of this size at this stage of development. The results of screening for B cells with the electron microscope were also compatible with this conclusion.

We conclude that the endocrine B cells are not derived from the neural crest. This conclusion may be extended to the other endocrine cells of the digestive tract, since Le Douarin and Teillet (*11*) in quail chicken heterografts were unable to find quail cells of neural crest origin among the chicken epithelial cells of the gut where various endocrine cells are present (*12*). In addition, since B cells do not originate from the neural crest, the characteristics of APUD cells cannot be used to determine neural crest origin. The origin of particular endocrine cells must be elucidated by other means such as the selective removal of the presumptive tissue of origin, by studies using embryonic mosaics, or by demonstration of specific molecular determinants (for example, membrane antigens). Our finding does not preclude an ectodermal origin for the endocrine cells of the gut, since these precursors may have colonized the endoderm earlier in development. Alternatively, they may have an endodermal origin: indeed, the concomitant development of both endocrine B and exocrine cells in the pancreas is consistent with the hypothesis of a common endodermal precursor for both types of cells.

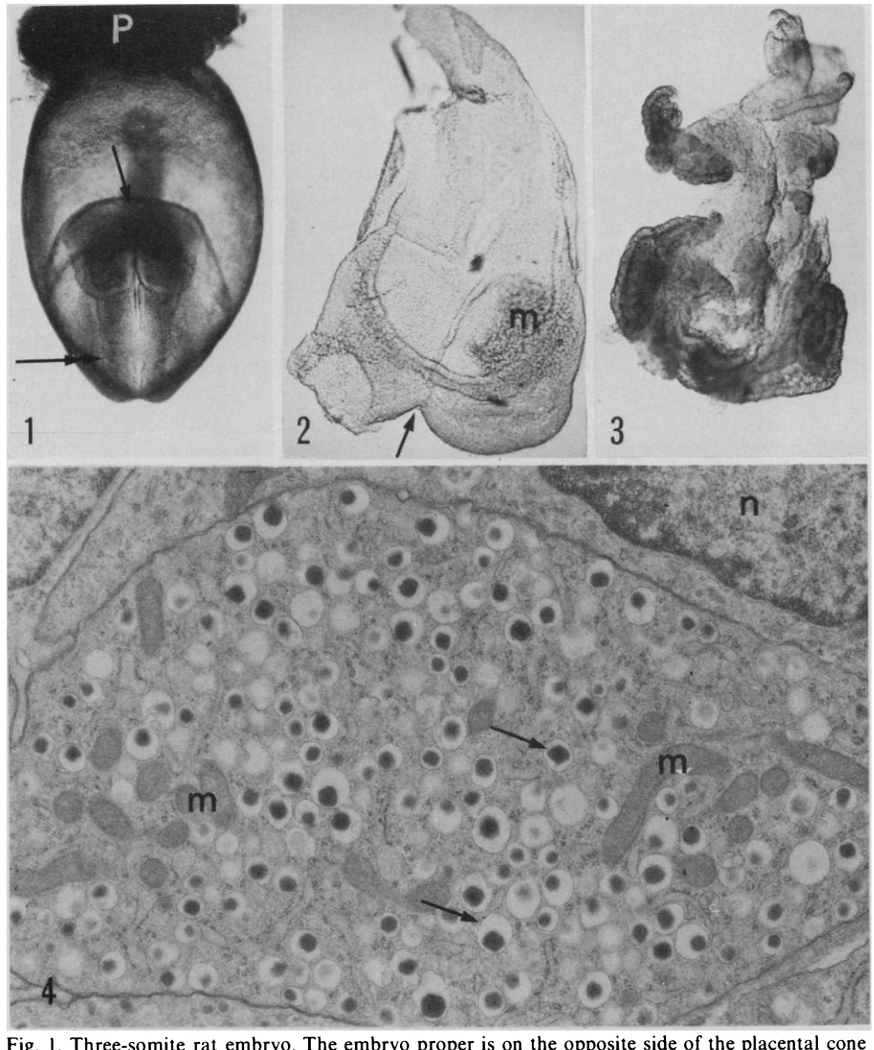

Fig. 1. Three-somite rat embryo. The embryo proper is on the opposite side of the placental cone (*P*). At this early stage the embryo is still inverted with the endoderm outside and the ectoderm inside. The head process appears as two dark masses, and the arrow indicates the depression formed by the neural groove. The first somites (double-headed arrow) are forming (× 25). Fig. 2. Ectoderm after removal by trypsin treatment. The shape of the head process separated by the forming neural groove (arrow) can be recognized. Some mesodermal cells (*m*) are removed with the ectoderm (× 60). Fig. 3. Mesoendoderm after removal of the ectoderm with trypsin. This is the tissue which is put in culture (× 60). Fig. 4. B cell in an embryonic rudiment explanted at three somites and cultured for 11 days after removal of the ectoderm. Granules (arrows) show the typical polygonal appearance of insulin granules; *N*, nucleus; *M*, mitochondria (× 11,000).

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