DEMONSTRATION OF ACID PHOSPHATASE-CONTAINING GRANULES AND CYTOPLASMIC BODIES IN THE EPITHELIUM OF FOETAL RAT DUODENUM DURING CERTAIN STAGES OF DIFFERENTIATION

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ABSTRACT

Dense cytoplasmic bodies surrounded by one or two unit membranes and containing mitochondria, vesicles, ribosomes, rough and smooth surfaced endoplasmic reticulum, and lamellated membranes (myelin figures) have been observed in the differentiating mucosa of the duodenum of rat foetuses by electron microscopy. Generally, the cytoplasmic components in the bodies seem to be in varying stages of disintegration. The bodies are found in greatest number on the 17th and 18th day of gestation, i.e. at the onset of differentiation. At this period of development the epithelium is stratified, and the villus formation is initiated by invagination of the epithelium by buds of mesenchyme followed by a splitting of the epithelium along the sides of the invaginations. When the villi have formed, the stratified epithelium has changed to the simple columnar type and the dense bodies have largely disappeared. Simultaneously, the lumen has widened considerably. In a parallel study with the light microscope, frozen sections incubated for the demonstration of acid phosphatase activity revealed the reaction product to be localized in bodies of the same size and distribution as the dense bodies found by electron microscopy. Hence, it seems that the bodies are altered and enlarged lysosomes (cytolysomes) active during the intensive differentiative events in the small intestine during the last part of intra-uterine life.

INTRODUCTION

Since the first studies on the histogenesis of the small intestine in mammals were published by Meckel (22) in 1817, the histological development of this organ has been the subject of many investigations (see 2, 8, 13, 17, 20, 48, 52, and review by Patzelt (38)). In the past decade histochemical and biochemical techniques have been applied in order to elucidate aspects of the functional differentiation (27-31, 40), particularly the activity of alkaline phosphatase during the development. In some of these studies (30, 31) the influence of exogenous corticoids was studied, and it was found that a precocious functional differentiation can be brought about by administration of hydrocortisone, which apparently only accelerates the differentiation, as no dissociation in the constituent events was observed.

By the introduction of the electron microscope a more profound information of the cellular differentiation in the intestine can be obtained. In this laboratory, electron microscope studies have for some time been undertaken to investigate the sequential events in the differentiation of the small intestine, and this paper deals with certain aspects
of the differentiation of the epithelium. Recently, it was published that membrane-bounded cytoplasmic bodies containing mitochondria, ribosomes, and endoplasmic reticulum were present in some of the intestinal epithelial cells in newborn rats (26). It was assumed that these bodies represent an early stage in the development of lysosomes. Bodies of this type are, however, far more numerous and much more complicated in structure during the last part of intra-uterine life when the formation of villi begins, and the differentiation of the intestinal epithelium into absorptive cells, goblet cells, and argentaffine cells is initiated. In the following account, these cytoplasmic bodies will be described and discussed.

MATERIAL AND METHODS

The duodena of albino rat foetuses of different ages were used in this investigation. Before and during pregnancy the mothers were kept under standard laboratory conditions. At the desired time of pregnancy the mother was lightly anaesthetized with ether, and phenobarbital in a dose of 4 mg per 100 g. of body weight was injected intraperitoneally. The duodena of the foetuses were exposed and removed. The placental circulation was kept intact as long as possible during this procedure. The tissue for electron microscopy was fixed in cold osmium tetroxide (37) in the modification given by Sjöstrand (46). Embedding was made in Vestopal W (44). Sectioning was done on an LKB ultramicrotome.

For the purpose of orientation, 1 to 2 micron thick sections for light microscopy were cut and stained with the cobalt sulfide method (25) or with basic dyes such as toluidine blue or basic fuchsin. Other sections were stained with pyronine, or the PAS reaction was carried out.

Thin sections for electron microscopy were contrasted for 1 to 2 hours with a 4 per cent aqueous uranyl acetate solution at room temperature (54). If a stronger contrast was desired, it was found advantageous to contrast the sections in a 2 per cent aqueous solution at 50°C for 30 minutes.

The sections were studied in a Philips electron microscope EM 100 B or a Siemens Elmiskop I.

For the demonstration of acid phosphatase activity in the light microscope, the duodena of rat foetuses 17 days old were fixed overnight in cold formol-calcium. Frozen sections were incubated at 37°C for 10 to 45 minutes in fresh Gomori medium (14) at pH 5. At-
tempts have been made to demonstrate the acid phosphatase activity, using the electron microscope, following the procedure given by Holt and Hicks (19), and by Miller (24). However, the ultrastructure of the sensitive embryonic tissues deteriorates very badly when incubated in the Gomori medium even at pH 6.2, and no study of detailed morphology has been possible so far.

OBSERVATIONS

1. Light Microscopical

The epithelium of the duodenum of a rat foetus on the 14th day of gestation is of the simple columnar type, limited from the surrounding mesenchyme by a well defined basement membrane. The columnar cells are very high with broad bases and tapering luminal ends, which limit the small, slit-like lumen. The nuclei are placed basally in the cells.

On the 16th day, small granules, which stain with toluidine blue and give a positive PAS reaction, are found in the apical cytoplasm.

During the following days an intensive mitotic activity takes place in the epithelium, resulting on the 18th day of gestation in a stratified epithelium consisting of four to six layers of cells. The lumen has widened considerably. Buds of mesenchyme (Fig. 1) forming the future connective tissue core of the villi are seen invaginating the epithelium. When the bud of mesenchyme has reached a certain height, the epithelium shows narrow clefts along the sides of the invaginations. In this way the first villi are formed. The epithelium has now reverted to the simple, columnar type.

Thus in certain stages of the differentiation of the mucosa of the duodenum, the epithelium changes from a thick stratified epithelium to a single layer of columnar cells. During this period of cellular rearrangement a variety of cytoplasmic bodies appear (Fig. 1) mainly in the cells bordering the lumen. The bodies are only occasionally found in the basal cell layers. Only a few are found on the 16th day, the numbers increasing on the following days and reaching a maximum about the 18th day. About 24 hours later they have largely vanished.

The bodies present a considerable variation in size, ranging from less than 1 micron to several microns. They are stained intensely by basic dyes, such as basic fuchsin and toluidine blue, but show no metachromasia with the latter in formalin-fixed
tissue. Some of the bodies contain PAS-positive material, and some are stained with pyronine.

Frozen sections of the duodenum of 18-day foetuses incubated in Gomori medium for the demonstration of acid phosphatase activity show a positive reaction in bodies of the same location and size as the dense bodies, but not all of the bodies do structure. Furthermore, bodies scarcely visible in the light microscope show up by electron microscopy.

In their luminal portion the epithelial cells show from the 14th to the 16th day an increasing number of vesicles of varying size (Fig. 3). The vesicles are bounded by a membrane of the same dimen-

![Figure 3](image_url)

**Figure 3** Electron micrograph of the luminal parts of several epithelial cells from the duodenum of a 15-day-old rat foetus. Numerous pinocytotic vesicles are seen. The vesicles are bounded by a membrane similar to the plasma membrane. At A an aggregate of vesicles is forming. × 35,500.

**Figs. 4 to 7** All figures are from epithelial cells of the duodenum of rat foetuses, 16 days old. L, lumen; A, aggregate of vesicles; T, terminal bar; M, mitochondrion.

**Figure 4** At A1 and A2 two vesicular aggregates are seen. In A2, besides the vesicles, ribosomes can be identified which presumably were included when the surrounding membrane formed. × 27,000.

react (Fig. 2). Heaviest accumulations of the enzyme reaction product are usually seen in the smaller bodies. Positive reaction was obtained only with incubation times of 20 minutes or longer, suggesting a rather low content of acid phosphatase in the reacting bodies.

2. **Electron Microscopical**

Examination with the electron microscope discloses that the cytoplasmic bodies observed in the light microscope have a varied and complicated structure. Furthermore, bodies scarcely visible in the light microscope show up by electron microscopy.

In their luminal portion the epithelial cells show from the 14th to the 16th day an increasing number of vesicles of varying size (Fig. 3). The vesicles are bounded by a membrane of the same dimen-
can be included in the vesicular aggregate (Fig. 4), showing the inclusion of granules, which probably are ribosomes. When the outer membrane of the aggregate has formed, the included vesicles show a content of varying electron opacity, and some of them contain still smaller vesicles. Thus the aggregates differ from the morphologically well defined multivesicular bodies (see 47 for review) in showing endoplasmic reticulum can be identified. Near the centre of the body a fine granular substance is seen. The relationship between the aggregates of vesicles and the dense bodies is uncertain, but it is thought that bodies like those shown in Figs. 6 and 7 may result if a condensation of a vesicular aggregate takes place. Mitochondria may also be included in the dense bodies as seen in Fig. 7.

![Figure 5](image1)

**Figure 5** This figure shows two vesicular aggregates (A₁ and A₂), each surrounded by a membrane. Some of the included vesicles show at this stage of development a content of a rather electron-opaque substance. Note the varying size of the included vesicles, and that some of them contain still smaller vesicles. The membrane of A₁ is not completely closed (arrow). X 24,500.

![Figure 6](image2)

**Figure 6** This body represents what is thought to be a further development—a condensation—of a vesicular aggregate. Parts of the rough surfaced endoplasmic reticulum have been included. Near the centre of the body a mass of finely granulated material is seen. X 20,000.

**Figure 7**

Greater variation in the size of the vesicles and in the content of other cytoplasmic constituents. Besides the vesicular aggregates, cytoplasmic bodies of a much more complicated type begin to show up in the epithelial cells of the 16th day duodenum (Figs. 6, 7, and 9). A common feature of these bodies is a surrounding membrane, which frequently is found to be double. Fig. 6 shows the luminal portion of an epithelial cell containing a dense body, in which vesicles, ribosomes, and rough surfaced apparently, the dense bodies can gain in mass by the apposition of additional layers of cytoplasmic components, the whole mass being surrounded by a new membrane. A comparison between Figs. 7 and 10 illustrates this conception. In Fig. 10, which shows a part of a large dense body, a dense central mass comparable to the dense body in Fig. 7 is seen. The central mass is bordered by a membrane, and is surrounded by a shell of cytoplasmic components in varying stages of disintegra-
tion, the body, as a whole, being delimited from the surrounding cytoplasm by two membranes. These are favourably cut only in the upper left part of the body.

The inclusion of a vesicular aggregate in a dense body is demonstrated in Fig. 9. A bell-shaped body is seen, which, in the upper more dense part, besides ribosomes and endoplasmic reticulum, contains a vesicular aggregate, the surrounding membrane of which is intact. The impression is gained that the body is about to engulf the fine filamentous substance seen in its center. This conjectured process of engulfing has been observed several times, and it is remarkable that the substance to be engulfed in every case has been the same filamentous material as shown in this figure.

It is an unsolved question, by what mechanism the membranes surrounding the vesicular aggregates and the dense bodies have been formed. It has been suggested that the fusion of vesicles could take place. Fig. 12 renders some support to this view, showing mitochondria, endoplasmic reticulum, and a dense granular mass surrounded partly by a double membrane, partly by a row of vesicles. The fusion of these vesicles would result in a membrane limiting the accumulation of cytoplasmic components from the cytoplasm of the cell. With the fusion completed, a body like that in Fig. 13 might appear. In this body nearly all kinds of organelles and inclusions that are found in normal cells have been surrounded by a double, bounding membrane. Four masses of roughly granulated material, presumably condensations of ribosomes, are seen (also shown in Figs 10 to 12). The included mitochondria show signs of dissolution. When the limiting membranes are double, the space between them is usually quite narrow, although occasionally some material may be seen in this space, as shown in Figs. 14 and 15. The two originally separate bodies in Fig. 14 have been enveloped by a membrane common to both. In the lower part of the body one mass of granular substance is
seen in the space between the membranes, some of the granules presumably being ribosomes. In the interior of the body, ribosomes, vesicles, and rough surfaced endoplasmic reticulum are seen, the endoplasmic reticulum partly surrounding two masses of coarsely granulated substance. The fine granular material (FG, Fig. 14) is also observed in Figs. 13 and 15; in the latter the contents of the body seem to be in a more advanced stage of degeneration.

Piles of parallel membranes are observed relatively often in the dense bodies (Fig. 13), and some bodies (Fig. 16) are built up solely by complicated membrane systems. In this body the membranes are, in places, arranged in “myelin figures” (Fig. 17). Other sections through this body have shown that the membranes seem to form a continuous system.

**DISCUSSION**

The heterogeneity of the cytoplasmic bodies described in this paper is clearly demonstrated in the electron micrographs presented. The bodies consist of an aggregate of different cytoplasmic components which are delimited from the rest of the cell by a single or double membrane.

The heterogeneity is largely dependent on the type and the amount of the included cytoplasmic components and the degree of dissolution of these.

As stated, the appearance of the cytoplasmic bodies coincides with a widening of the intestinal lumen, the formation of villi, and the onset of differentiation of the epithelial cells. It seems natural to think that the bodies represent a mechanism for isolating and hydrolyzing cellular waste products resulting from these processes.

Tentatively, based on the static pictures the micrographs represent, a line of development of these bodies may be advanced. It is assumed that the vesicular aggregates (Figs. 3 to 5) and the bodies seen in Figs. 6, 9, 12 to 14 represent initial and early stages, and Fig. 16, a terminal stage in such a hypothetical sequence of transition. The other figures represent various intermediate stages.

Lipids and lipoproteins show a tendency to arrange themselves in “myelin figures” in watery media (18, 50), and the myelin figures shown in

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**Figure 9** This figure shows a body which on section has a horseshoe-like appearance. The “shoe,” which seems to be closed (at the lower right the inner and outer membrane show continuity (arrow)), contains a vesicular aggregate (A), ribosomes, and endoplasmic reticulum. One gets the impression that the body is in a process of engulfing the fine filamentous material (F). (See text). × 40,000.
Figs. 13 and 16 are thought to consist of lipids or lipoproteins released in the process of the breaking down of organelles included in the cytoplasmic bodies. That the lipids released by the digestion of erythrocytes in spleen macrophages form myelin figures was demonstrated by Stoeckenius (49), and similar myelin figures and lamellated bodies have been observed in a variety of cells under physiological as well as pathological circumstances (6, 21, 39, 16, 23, 55).

The finding of cell deaths in embryological processes, such as the formation of lumina in solid glands, histological differentiations, and regression of transient structures has been reviewed by Glücksmann (15). The transient occlusion of the lumen of the duodenum at places during development has been described in man by Forssner (13) and Johnson (20) and in the rat by Tandler (52) and Forssner (13). In this material the lumen of the duodenum has not been found totally occluded. 1 to 2 micron thick sections of Vestopal-embedded tissue have shown that a very narrow lumen is present, although it is easily overlooked in ordinary paraffin sections. By following the morphological criteria of cell death given by actual process of phagocytosis, however, has not been observed. Bellairs (3) has described in a combined light and electron microscopic investigation of cell death in chick blastoderm, dense bodies very similar to the bodies presented in this paper, and considers them to be degenerating and phagocytosed cells. The "banding of granules" described by Bellairs (3) in degenerating cells has not been observed in this material. Likewise, Cohen (7) has found similar dense cytoplasmic inclusions in the tissues of the head of 8-to-9-day mouse embryos, and has suggested their origin from phagocytic activity.

In a study of the cellular differentiation of the
kidneys of newborn mice, Clark (6) found large, PAS-positive cytoplasmic bodies in the differentiating proximal tubular cells, some of which showed dense, concentric lamellae and altered mitochondria, besides some amorphous material. Clark pointed out the possible relationship between these cytoplasmic bodies and vacuoles and small canals also found in the cytoplasm. Similar cytoplasmic bodies have been observed by Novikoff (32) in the proximal tubular cells of rat kidneys after ligation of the ureter. These bodies were identified with acid phosphatase-rich bodies seen by light microscopy. In isolated rat livers perfused with glucagon, Ashford and Porter (1) found membrane-bounded bodies containing cytoplasmic components in various stages of breakdown. These authors suggest that the bodies represent areas of autolysis that have been encapsulated by a membrane to protect the rest of the cell.

In an investigation of the influence of folic acid antagonists on the human proximal intestine, Trier (33) found a coincidence between the mitotic inhibition in the crypt epithelium and the appearance of spherical bodies in the cytoplasm of many crypt cells. In the electron microscope these bodies showed a morphology identical to that of many of the bodies seen in the foetal duodenal epithelium. It is interesting that Trier found the bodies confined exclusively to the epithelial cells of the crypts: they were never seen in the differentiating villous epithelial cells and only occasionally found in the crypt lumen.

The ultimate fate of the dense bodies found in this material is just as obscure. As previously stated, they have largely disappeared when the first small villi have formed, and only on rare occasions have been seen in the lumen and in the above-mentioned epithelial clefts. Possibly, as also suggested by Glücksmann (15) and other authors (53, 1, 45), the breakdown products of the contents of the bodies may be utilized again.

The finding of acid phosphatase activity localized in cytoplasmic bodies in the luminal portions of the epithelium in parallel studies with the light microscope classifies at least some of the bodies in the group of lysosomes.

The acid hydrolases localized in the lysosomes (9) have as substrates many important chemical compounds of the cell, and it has been suggested (9, 10) that the lysosomes may act as miniature digestive systems capable of breaking down endogenous cell constituents as well as phagocytosed exogenous material. The activity of lysosomal hydrolases in homogenates of regressing Müller ducts of the chick embryo has been studied by Brachet et al. (5), who found a marked increase in the unsedimentable activity of acid phosphatase coincident with the regression. Recently the term “cytolysomes” (33, 34) has been proposed for the enlarged and altered lysosomes, which have been observed in cytolysing cells, under both physiological and pathological circumstances (35, and reviews by Novikoff 33, 34). Possibly, the dense bodies described here should be grouped under the headings of cytolysomes as well as phagosomes (33, 34, 51, 11).

It is an open question how the acid phosphatases have originated in the bodies. The small granules found in the apical cytoplasm of the epithelial cells on the 15th to the 16th day, and which stain with toluidine blue and give a positive periodic acid-Schiff reaction, are presumably

Figure 11  This cell contains three cytoplasmic bodies, two of which are very dense (DB). The membrane surrounding the dense bodies is not very well defined, but can be seen in the lower body (arrow). Presumably, the granular content consists of ribosomes. Dense bodies of this kind are often found at this period of development. N, nucleus. X 18,500.
Figure 12 This figure shows masses of ribosomes, parts of the endoplasmic reticulum, mitochondria (M), and a rounded mass of granulated material partly surrounded by a membrane (ME), partly by a layer of vesicles (arrows). It is supposed that a limiting membrane may form by fusion of the vesicles, and that a cell in this way can isolate unwanted substances from the cytoplasm. N, nucleus. X 24,500.

Figure 18 This large body is bounded by two membranes. The broken outer membrane at the lower right is probably an artefact. Nearly all kinds of cell organelles are represented. Four masses of granular material resembling the dense bodies in Fig. 11 are seen. One of the masses is partly surrounded by a membrane (arrow). At LM a pile of short, parallel membranes is seen. The fine granular substance (FG) is also seen in Figs. 6, 10, 14, and 15. P, nuclear pores. X 18,000.
identical with the aggregates of vesicles seen in the electron microscope. As stated, vesicles and aggregates of vesicles are seen incorporated in dense bodies, and the stepwise transformation of vesicular aggregates to dense bodies has been suggested.

Vital staining procedures have not been performed on this material. Argeseanu and May (2), however, observed “small elements” in the cytoplasm of the epithelial cells of the small intestine which seemed to contact newly formed pinocytotic vacuoles. After a certain number of contacts with microkinetospheres, the pinocytotic vacuoles changed their refractive index and became stainable with neutral red. Rose suggested the microkinetospheres to be identical with lysosomes. Recently, Ogawa et al. (36) have demonstrated that neutral red granules in cell cultures of fibroblasts and astrocytes contain acid phosphatase of chick embryos. The small elements increased in number towards the 15th to 16th day of incubation when the differentiation of the epithelium begins. The small elements were impregnated by silver techniques and stained vitally with neutral red.

Brachet (4) has suggested that lysosomes are identical with the “neutral red granules” which are found in many cells after vital staining. In a study of rapidly growing cultures of HeLa cells with phase contrast cinematography, Rose (43) observed small granules (microkinetospheres), and correspond to lysosomes. This fits well with the earlier observation that phagocytosed cellular inclusions are usually strongly stained vitally with neutral red (42).

If the bordering membrane of the bodies originates by the fusion of vesicles (Fig. 12), enzymes may also be provided to the bodies in this way. Recently, Miller (24) has demonstrated in the electron microscope that the reaction product of acid phosphatase activity in renal protein absorption droplets is “very precisely localized along the entire inner face of their bounding membrane.”
FIGURE 15 As in the bodies in Figs. 13 and 14, this body is bounded by two membranes. A flocculated substance (FS) and wrinkled membranes (VM) have been included between the membranes when the outer one formed. Again, disintegrating mitochondria, ribosomes, vesicles, and fine granular substances are seen (FG). × 34,000.

He also found small amounts of the reaction product in the tubular invaginations.

Essner and Novikoff (12) in a study on human hepatocellular pigments observed that growing lipofuscin granules, which they consider to be altered lysosomes, have the acid phosphatase activity restricted to the periphery of the granule.

These findings are thought to give some support to the previous statement (under "Observations") that the dense bodies may enlarge by the apposition of new layers.

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This figure presumably represents a final stage in the breaking down of the contents of the bodies, showing a complicated membranous system, arranged in places in myelin-like figures. Sections at other levels of this body showed continuity between membranes which in this section appear closed or seem to end abruptly. X 39,500.

A higher magnification of the myelin-like figure in Fig. 16. X 79,000.


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