Prolonged osmification as an indicator of the differentiation process in the endomembrane system

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ABSTRACT The technique of prolonged osmification was used in the analysis of reducing capacity of perinuclear space, endoplasmic reticulum and cis-Golgi cisternae in different epithelial cells during embryonic differentiation and immediately after the birth. Cells of the mouse gastric and intestinal epithelium and of the exocrine pancreas and mammary gland were analyzed. It was shown that endomembrane compartments exhibit high variability in their capacity to reduce OsO_4 into lower valency oxides. Typical staining of cis-Golgi cisternae by osmium black does not occur before the cells achieve the developmental state in which production of specific products starts. The changes in stainability occurring from the perinuclear space and endoplasmic reticulum towards the cis-Golgi cisternae indicate a maturation pathway with no direct correlation to the chemical characteristic of the substances produced in different cell types. In the mammary gland the reduction capacity of endoplasmic reticulum disappeared with the intensive synthesis of lipids. Considering our previous results and those of other authors, the possible reasons for the observed dynamics in reducibility in particular segments of endomembraneous space are discussed.

KEY WORDS: *prolonged osmification, reducing capacity, endomembraneous compartments, differentiation, epithelial cells*

Introduction

Based on our previous work on the complex carbohydrates in the developing glandular cells, here we investigate further into the system where these products are formed (Pipan and Psenicnik, 1985). Our results achieved on mucoid cells (Psenicnik and Pipan, 1989) and cells in which the normal rhythm of secretion was modified by secretagogues (Jezernik and Pipan, 1989) directed our next investigation into comparative analysis of endomembraneous compartments showing the reduction capacity for OsO_4.

The prolonged osmification method is derived from the so-called metallic impregnation technique used by Golgi and others in light microscopic staining methods (Whaley, 1975). This method is based on the reduction of OsO_4 to the insoluble lower valency Os-oxides, Os-black. Although prolonged osmification is still considered as the method which specifically stains the cis-Golgi vesicles (Friend and Murray, 1965; Samuel and Flickinger, 1987), Locke and Huie (1983) have achieved the staining of perinuclear space, endoplasmic reticulum and Golgi vesicles by its modification using OsKJ. In their opinion, the reacton would detect labile S-S bridges of proteins as they are processed along their glycosylation pathway. This was important for our investigations because the method offers information on the characteristics of the endomembraneous system where specific glycoproteins are formed and processed. The fact that unmodified Os impregnation reveals a different reduction capacity inside the endoplasmic reticulum and Golgi apparatus was the reason for its application (Friend and Murray, 1965) in our present investigation of different mouse epithelial cells on the ultrastructural level.

Cell selection was based on their secretory activity. In all cases we used the cells during differentiation. Namely, remarkable morphological alterations occur 4 to 5 days before the birth of the mouse. The changes appearing during the fetal development can be compared to the changes during continuous removal of epithelial cells along the crypt-villus axis in the small intestine and gastric epithelial cells in the adult animals. Besides these two different tissues the cells of the exocrine mouse pancreas and mammary gland cells have also been analyzed. The study included embryos and adult mammary glands.

Observations

The general results of our comparative investigation can be summarized in the statement that the presence of Os black, which indicates the reduction capacity of a given endomembraneous compartment, varied not only in different cell types but also within the same cell type. It also allows the anticipation of some rules.

In the stratified gastric epithelium of 14-day-old embryos, Os black was observed in the perinuclear space and in the poorly developed rough endoplasmic reticulum, while the Golgi complex

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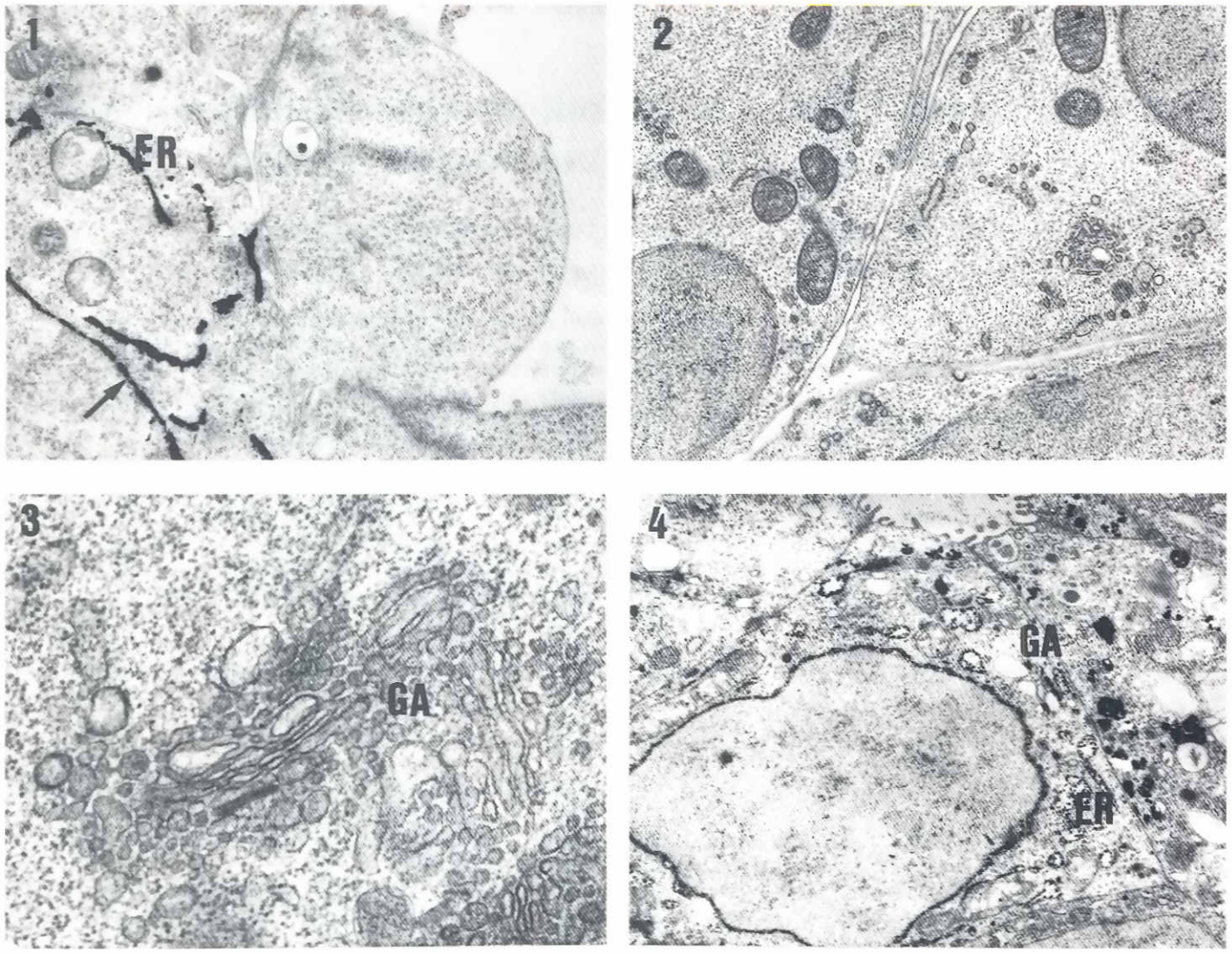

Fig. 1. After prolonged osmification Os-deposit is found in the perinuclear space (arrow) and in the poorly developed endoplasmic reticulum (ER) in the gastric cell of 14-day-old mouse embryo. *x17000.*
Fig. 2. Undifferentiated cells of the small intestine do not show any Os staining. *x19000.*
Fig. 3. Os staining of the pancreas acinar cell of 15-day-old embryo. *There is no staining of the Golgi apparatus (GA). x46000.*
Fig. 4. Differentiating alveolar mammary cell, the 15th day of pregnancy. *Os black is deposited in perinuclear space, luminal side of endoplasmic reticulum (ER) and cis-Golgi apparatus (GA). x10000.*

remained completely unstained (Fig. 1). Undifferentiated small intestinal cells did not show any deposition in the endomembraneous compartments (Fig. 2). Similar results were also obtained on the cells of the exocrine pancreas, where on the 14th day of gestation Analysis of the cells between days 17 and 19 showed the sudden changes in the reduction activity of their endomembraneous system. A similar reduction capacity was observed also in neonatal animals. When the formation of secretory granules has turned on in the gastric epithelium, the typical cis part of the Golgi complex could be identified (Fig. 5), while the osmiophily of the perinuclear region and endoplasmic reticulum became irregular (Fig. 6). High reduction capacity in the cis-Golgi and numerous small vesicles is also observed in the parietal cells just after birth (Fig. 7). An interesting detail in the endomembraneous reduction capacity was revealed in the cells of the exocrine pancreas, where at the 17th day only vesicles estimated as transition vesicles between the endoplasmic reticulum and the cis-Golgi are filled with osmium black (Fig. 8). When the stained vesicles at the cis-Golgi side appeared on the 19th day, the cells achieved the step typical for the cells of adult animals. A somehow intermediate situation has been observed in the cells of the small intestine. In some column cells, especially those located near the top of the forming villus, the endoplasmic reticulum was intensively stained in contrast to the Golgi region where only a few cisternae had Os deposits. Towards the end of

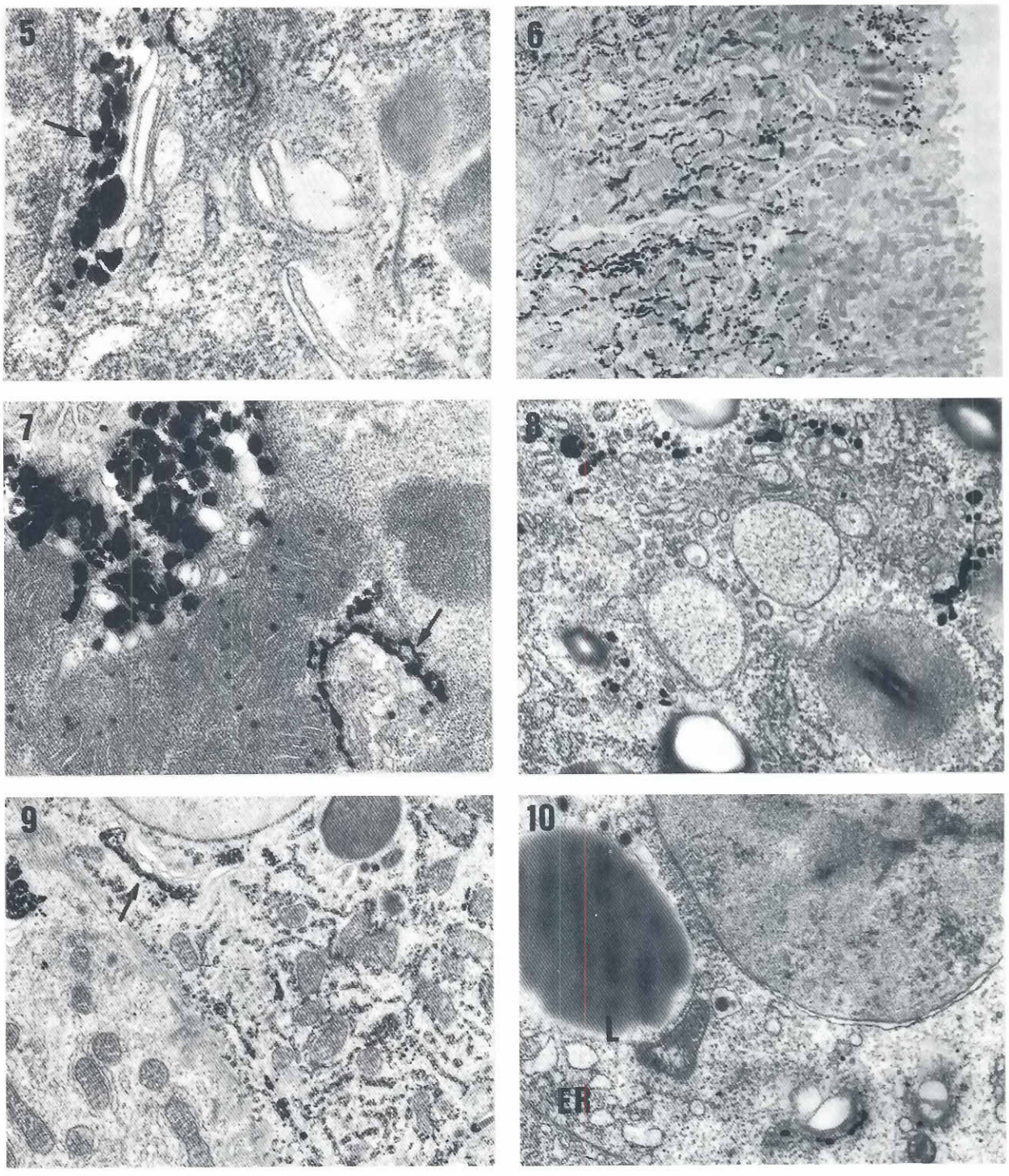

Fig. 5. One day after the animal birth the highly stained cis-Golgi part (arrow) can be observed in the cells of the stomach epithelium. x40000.
Fig. 6. Osmiophily in the gastric cells of neonatal animals varies from cell to cell. *x5500.*
Fig. 7. Os-black is often observed in the cis-Golgi part (arrow) and in the numerous small vesicles in the parietal cells of neonatal animals. *x31000.*
Fig. 8. Os staining of pancreatic acinar cell of 17-day-old embryo is localized in numerous transitional vesicles. *x27000.*
Fig. 9. Heavily stained cis cisternae of the Golgi complex (arrow) and endoplasmic reticulum appear in the cells of the small intestine just after the animal birth. *x9000.*
Fig. 10. Prolonged osmification of alveolar mammary cell on the 1st day postpartum gives no positive reaction on the membranes of the endoplasmic reticulum (ER). *Some Os black deposition is seen in the cis-Golgi part (L-lipid droplets). x10000.*

gestation heavily stained cis cisternae also appeared (Fig. 9). In the mammary gland, the strong reduction capacity found in the cells during gestation disappeared when lactation started (Fig. 10).

Concluding remarks

Our results confirm our expectation that prolonged osmification could expose the somehow hidden difference within morphologically undistinguishable endomembraneous compartments of different developmental stages and different cell types. The variations included all parts of these systems: perinuclear space, cisternae of rough and smooth endoplasmic reticulum and, most interesting, also of the cis part of the Golgi complex. It is well known that the Golgi complex is characterized by a morphological polarity which corresponds to the cytochemical polarity (Novikoff and Goldfischer, 1961). The enzyme thiamine pyrophosphatase (TPP-ase) and cytidin monophosphatase (CMP-ase) are preferentially located on the trans side. The nicotinamid adenosin dinucleotide phosphatase (NADP-ase) was detected in the midsaccules, while prolonged osmification is considered as selective labeling of GA of the cis face (Friend and Murray, 1965). Our results clearly indicate that prolonged osmification cannot be the absolute valuable indicator of the immature face of a Golgi stack, as is usually believed (Samuel and Flickinger, 1987; Rambourg and Clermont, 1990). However, it can be taken as a somehow specific indicator of reduction capacity of any part of the endomembraneous vacuolar space. The main question arising from our results concerns more or less specific changes in the behaviour of a particular segment of the endoplasmic reticulum or Golgi stack.

The most common finding in analyzed epithelial cells was the lack of Os black in Golgi stacks of relatively undifferentiated cells. Such a result strongly suggests that this situation reflects morphological and functional immaturity. The osmiophily of endoplasmic reticulum and perinuclear space before the cis-Golgi part could have something to do with the «maturation» of products flowing in the vectorial process from the endoplasmic reticulum to the cis-Golgi compartment. A correlation between the maturity of secretory products and the staining ability of the cis-Golgi cisternae suggests also the observation of the transitional vesicles involved in «transport» of osmium black to the immature Golgi face in the cells of exocrine pancreas observed on the 17th day of embryonic development. Later on, the cis cisternae always contain Os deposits. The gradual increase in osmium black deposit along the crypt-villus axis supports such a possibility; namely, the reduction capacity increases towards the upper part of the villus where the mature cells arising from the undifferentiated crypt ones are finally placed after the migration.

At least two questions have remained unanswered. First, which agent is involved in OsO_4 reduction; and second, what is the explanation for the difference in the reduction ability of morphologically identical cells presumably on the same developmental level and lying not far apart from each other. In all cells investigated here, there is undoubtedly a correlation between synthetic activity and the ability to reduce OsO_4. There remains, however, the unsolved problem of the existence of one or several chemically defined substances which would possess this reduction potential. Different cells whose common characteristic is producing different secretory products have a similar response. Obviously the reducing ability depends only on, more or less comparable, steps of achieved secretory capability. In our analysis of maturation of secretory granules in the mucoid cells (Pipan and Psenicnik, 1985) and reduction capacity in the same cells (Psenicnik and Pipan, 1989) we tried to interpret our results with biochemical data of Bischoff and Kornfeld (1986). The absence of osmium black of less differentiated mucoid cells could be the consequence of the lack of functional composition and activity in mannosidases of not yet differentiated cells. However, we could not establish the direct relation between mannosidase and reduction capacity. Because of the suggestion of Lodish *et al.* (1987) that the tertiary and quaternary protein structure is the signal for the exit of glycoprotein molecules from the endoplasmic reticulum to the cis-Golgi side, the existence of labile S-S bridges of unrevealed protein on their glycosylation pathway, as was proposed by Locke and Huie, became somehow questionable.

The second open question referring to the difference in the reduction capacity observed in morphologically and functionally similar cells found previously in the small intestine could be explained by the results of De Rubin *et al.* (1989). The expression of 5 genes was followed during embryonic development. It was found by immunocytochemical methods that different products could be identified in neighbouring cells. Therefore, a mosaic staining pattern arose. Although our results are not directly comparable to those of De Rubin *et al.* (1989) the mosaic pattern in the reduction ability of developing intestinal cells seems to be more understandable.

The results obtained on the mammary gland may be similar to the other epithelial cells. At the beginning of lactation, a prominent increase in lipid synthesis occurs along with the synthesis of casein and lactose. Correlation between intensified lipid synthesis and decreased reducing power of the endoplasmic reticulum and the cis-Golgi complex suggests that the reducing power of endomembranes could be associated with the amount of reducing equivalents or with the activity of redox enzymes. Microsomal fatty acid desaturase activity increases during mammary gland differentiation (Rao and Abraham, 1974) and this enzyme complex comprises also cytochrome b reductase, which could possibly reduce OsO_4. Jarasch *et al.* (1977) also mentioned that xanthin oxidase, an indicator of secretory differentiation in mammary cells (Ringo and Rocha, 1983), can function as «NADH oxidase» and «NADPD oxidase». The decreased reducing power of endoplasmic reticulum membranes could also be assigned to the lowering of NADH and NADPH concentrations.

Although our research is at some points hypothetical, it clearly shows that prolonged osmification has revealed previously unknown dynamics and specificity of the endomembraneous system during the differentiation process, concerning the way its specific products are synthesized.

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