IMAGE PROCESSING AND NUMERICAL ANALYSIS APPROACHES OF POROSOME IN MAMMALIAN PANCREATIC ACINAR CELL

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Abstract: Pancreatic acinar cells have a particular role in the synthesis, storage and regulated secretion of the different digestive enzymes present in pancreatic juice. Recently a detailed understanding of the molecular machinery and mechanism of cell secretion has come to light. So, it is now an accepted belief, that the mechanism of cell secretion is quite different, and a highly regulated process. The secretory vesicles (zymogen granules) dock and transiently fuse at the base of specialized plasma membrane structures called porosomes or fusion pores. In the present study, TEM images and a computer-assisted morphometry approaches have been used to analyze quantitatively the shape and fine structure of porosome in pancreatic acinar cell of post-feeding rat. The electron micrographs illustrated the porosome as a distinct permanent, lipoprotein, cup-shaped structure at the cell plasma membrane of the pancreatic acinar cell, facing the lumen and varies in shape and structure according to the stage of secretion. The computer-assisted morphometry summarized the structural and morphological importance of the porosome and revealed its different shapes during the cell secretion. The coloured images have clearly shown the supramolecular tripartite lipoprotein structure of the plasma membrane and porosomes. Histographic analysis showed the numerical values of the secreted materials and the three layers of both porosomes and plasma membrane which differed depending on the stage of cell secretion.
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1. Introduction:

Since last decade, secretory process in the cells attracted great attention and concern of several investigators, and a series of elegant studies revealed a completely different molecular mechanism of secretion and membrane fusion in cell as a very important process involved in the release of hormones and digestive enzymes (Jena *et al.*, 1997, 2003; Cho *et al.*, 2002a, b, d,&e; Jeremic *et al.*, 2003; Craciun, 2004; Kelly *et al.*, 2004; Thorn *et al.*, 2004). These authors demonstrated that pancreatic acinar cells have a particular role in the synthesis, storage and regulated secretion of the digestive enzymes present in pancreatic juice. The process of pancreatic secretion involves the docking and transient fusion of membrane-bounded secretory vesicles (the zymogen granules) at a specialized plasma membrane structures called porosomes or fusion pores, to discharge vesicular contents. In 1997, Schneider *et al.* represented the first discovery of the porosome as a circular pit containing 150 nm diameter depression, presents at the apical cell plasma membrane where secretion occurs in living pancreatic acinar cells. Also, Jena who is considered as one of the most pioneer investigators in the studies of porosomes elucidated singly a lot of searches in (1997; 2002; 2003; 2004; 2005; 2007; 2008; 2009), in addition to his work with other teams. In his interesting and intense studies of the story of cell secretion, he elucidated the porosome as a permanent universal secretory machinery in a nanometer-size lipoprotein structure at the cell plasma membrane. In addition, determination of porosome biochemical composition; its functional reconstitution into architecture lipid membrane; its structure and dynamics at nm resolution and in real time have been all explained by Jena *et al.*, 2003; Jeremic *et al.*, 2004a,b; Lee *et al.*, 2004, 2009; Cho and Jena, 2007; Cho *et al.*, 2007, 2008; Cook *et al.*, 2008; Potoff *et al.*, 2008. The monumental discovery of the porosome as a new cellular structure at the plasma membrane and its molecular mechanism of secretion were also described in the reviews of Anderson (2004; 2006) and Zhvania (2004). Similarly, Craciun (2006) and Jeftinija (2006) determined in their interesting studies the structure of the porosome in resting pancreatic acinar cells and when co-isolated with the secretory vesicles in exocrine pancreas (the zymogen granules), and demonstrated the presence of porosomes at the apical plasma membrane where secretion occurs. Ultimately solving one of the most difficult, significant, and fundamental cellular process – the cell secretion.

On the other hand, image processing is a technique for processing any image, it depends on the fact that the causes of colour in many structures are in response to the structural irregularities (Fortner and Meyer, 1997; Fraser *et al.*, 2003; Gendler, 2003; Rector *et al.*, 2004) Such use of this property can be considered as the key factor for mapping the way in

which the electron beam of TEM interact with the internal structure of organelle to produce the digital image. This digital image was further used for better characterization of the differences in fine structural field. However, the digital image consists of a square array of image elements or pixels; at each pixel, the image brightness was sensed and assigned with an integer value (from 0 to 255 in the case of gray scale image) that was named as the gray-level. For better visualization of the image, the gray-level image is transformed into colour image and converted into hue, saturation and intensity (HIS) using a discoloured technique. The simplest way of obtaining a pseudo colour image from a gray-level image is to use the RGB mode. An RGB colour consists of three individual images exposed through Red, Green and Blue filters, which are eventually combined into a single composite colour image. Note that, the individual RGB images are not in colour. They will still be gray scale images until combine them into the final colour image. This is recognized by many of the popular image processing programs like Photoshop or Paint Shop Pro (Parker, 1997; Sonka *et al.*, 1998; Myler, 1999). These programs are excellent tools when you want to crop, resize and perform final adjustments to your colour images and hence. Also, it can offer both more feasible and practical performance at simple tasks and good implementation, which would be impossible by TEM or other tools alone. However, the purpose of RGB colour model is to facilitate the specification of colours in some standard, generally accepted way, and is the most commonly used model in graphics devices (MacDonald, 1999; Lynch and Livingston, 2001). This model however, allows offering colour range for the pixels from an integer value 0 to 16777215 (number of colours: 256x256x256). This can be used as an additional parameter for identifying the fine details of the differences in the ultrastructure features.

On the light of this, the present study has been carried out to visualize on the nanosize structure and analyzes numerically and histographically the porosomes ultrastructure in mammalian pancreatic acinar cell.

2. Materials and Methods:

2.1. Experimental Animals and Ultrastructural Preparation of Pancreatic Acinar Cells:

Ten male albino rats (*Rattus norvegicus*) ranging in weights from 150-200g were acquired from Schistosoma Biological Supply Program (SBSP) Theodor Bilharz Research Institute, Cairo, Egypt. Housed in clear plastic cages (one rat/cage) with wood chips as bedding and given pellet rodent diet, in addition of milk and water *ad-libitum*. They were kept for acclimatization and observation of their eating habit for a week, under controlled environmental conditions, including a temperature of 25^0C and a 12h light/ dark cycle. At the last day, after they finished eating presented meal at morning, five rats were sacrificed by decapitation one hour post-feeding, while the others were decapitated two hours post-feeding. The pancreas were rapidly excised and were processed for ultrastructural evaluation by electron microscopy as described previously by Dykstra *et al.*(2002) as follows: Freshly excised pancreas were cut into small blocks ($1 \times 1mm^3$) fixed in cold 4F:1G (i.e. 4% formaldehyde and 1% glutaraldehyde adjusted at pH 2.2) for 24h, and postfixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.3), dehydrated in an ethanolic series culminating in 100% acetone, and infiltrated with epoxide resin. After polymerization overnight at 60^0C, semithin section (0.5µm) were stained with 1% toluidine blue in 1% sodium borate and examined with a light microscope. Areas of exocrine acinar cells were selected and the blocks trimmed accordingly. Ulrtathin sections (80-90nm) were cut, placed on 200 mesh copper grids, and stained with uranyl acetate and lead citrate. The grids were examined and photographed using JEOL.JEM-1400-EX-ELECTRON MICROSCOPE at the Central Laboratory of Faculty of Science, Ain Shams University, Cairo, Egypt. The photographs were printed on KODABROMIDE F5s GLOSSY Black and White- Schwarzweib-Kodak.

2.2. Computer-assisted Examinations:

The TEM images of porosome at the plasma membrane of pancreatic acinar cell were visualized and examined by applying Cartographic Information System software (CIS) technique (Shulei and Yufen, 2004). Combination of image processing; numerical analysis; artificial intelligent and expert system with general vision software were used to colourize; analyze and reveal the morphological and Ultrastructural changes in porosomes by using Adobe® ImageReady® CS Middle Eastern Version "8".

3. Results and Discussion:

The electron micrograph of one hour post-feeding rat, of the apical region of the pancreatic acinar cell displays clearly the presence of porosome as a swelled flask-shaped structure at the cell plasma membrane. The mouth of the porosome opening to the outside, range in size from 130 to 150 nm in diameter. It is loaded with electron dense particles at its groove. Some of these dense particles (enzyme materials) are also seen at the acinar lumen, as shown in figure (1).

TEM micrograph of two hours post-feeding period (Fig.2) illustrates that the porosome is more or less cup-shaped with wider mouth opening (range in size from 250 – 300 nm), compared with that seen at one hour post-feeding in figure (1) and no dense particles can be detected at the groove of this porosome, i.e. the secretory material was discharged, while the acinar lumen is loaded with these dense

particles. Also, the plasma membrane appears in these electron micrographs (Figs.1&2) as a triple-layered structure consisting of two dense bands separated by a central clear zone of lipid. The structure of plasma membrane shown in these electron micrographs coincides with the well known nomenclature "the unit membrane" or the "tripartite structure" that suggested and established by Robertson (1959; 1960b) as referred in the interesting book of Heimburg (2010).

Image analysis of these electron micrographs (Figs.1&2) after being processed with CIS and histographic analysis clearly demonstrate an obvious appearance of the tripartite lipoprotein structure of the plasma membrane as; an outer and an inner protein layers which are coloured with blue colour, while the lipid bilayer that sandwiched between them is coloured in yellow, as clearly seen in figures (3A –5A) that represented one hour post- feeding period and in figures (6A- 8A) which represent two hours post- feeding period. The porosomes in these figures also appear with the same colouration of plasma membrane on their three lateral boundaries of the cup-shape which emphasize that porosome is originated from plasma membrane as a permanent supramolecular lipoprotein structure as reported by Jena (2008). In addition, it is worthy to mention that the fluid mosaic model of Singer and Nicolson (1972), is clearly seen in the coloured figures (3A-5A), as the membrane lipid is arranged predominantly in the form of a bimolecular layer which is frequently interrupted by the presence of integral embedded protein extended from the protein layers in the form of mosaic arrangement.

Also, Singer (1992) and Lundbaek *et al.* (2010) established that the lipid bilayer is essentially a fluid substance permuting lateral mobility of both the lipid and protein molecules, and hence they are capable of transitional movement within the whole bilayer. The coloured figures (6A-8A) reveal this movement by the changeable features of plasma membrane layers and porosome in comparison with the figures (3A-5A) after releasing of the enzymatic materials in to the lumen which appear loaded with these materials in the period of two hours post- feeding. That means, the plasma membrane layers and porosome are essentially involved in the process of cell secretion.

In the same coloured figures (3A-8A) the image analysis of the electron dense materials which are seen at figures 1&2 in the groove of porosome cup appear with a distinct colour of deep green, which represent the enzymatic materials, and the released enzymes from this porosome in the lumen have the same green colouration.

Regarding histographic analysis, it is well known in CIS technique as reported by Rector *et al.*, 2004; Shulei and Yufen, 2004, that the colour which is appeared, is not randomly colour, but it is related to the formation of the structure, i.e. it means that the constituents of the structure is formed of its individual elements in different colours which combine and give the specific colour of the structure.

The histographical analysis is formed of two dimensions (2D); the X and Y axises. In this study, the Y axis represented the amount or the concentration (quantitatively) of the elements or compounds, whereas X axis represented the different elements or compounds of the structure.

Histographical analysis of the coloured pictures of the lipoprotein layer of plasma membrane and porosome, in addition to the enzymatic material are represented in figures (3B-8B).

It is obviously noticed during comparison between the figures (3B-5B), which represent the features of one hour post-feeding period and the figures (6B-8B), which represent the features of two hours post-feeding period, the quite differences in arrangement and concentrations of the protein layers and lipid bilayer of the plasma membrane and porosome, as well as enzymatic material, i.e. the position of green, red, blue in the elementary map of protein and lipid layers and enzymatic material appeared totally of varying degrees of concentration and accumulation from one hour to two hours post – feeding periods. This means that the lipoprotein layer of plasma membrane and porosomes are in active and changeable condition during the process of secretion to allow the transformation of the secretory materials (the enzymes) from the inside of the pancreatic acinar cell to the outside into the lumen. This can be obviously detected by the elucidation of the histographical figures.

As seen clearly in coloured figure (3A) of one hour post- feeding period, the inner and outer protein layers which have a distinct architecture and are coloured in blue and marked in white dots have a value of 53.96 and pixelation area is 8477 pixels in the histogram (Figure 3B). The arrangement of the peaks of this histogram are green, red and blue, which may reflect that they are different types of amino acids or protein constituents of the plasma membrane. While the same layers of protein in figure (6A) which represent two hours post-feeding period, appear totally different in arrangement and it looks like that they are involved in the secretory process. Their histogram analysis in figure (6B) show a value of 101.53 and the pixelation area is 16637 pixels. The arrangement of the peaks of this histogram are also green, red and blue, but in different concentrations and a little far distance of each others.

For the lipid layer, at one hour post-feeding period, it obviously appears in figure (4A) as a distinct bimolecular layer having variant degrees of deep yellow colouration and the marked white dots have a value of 82.69 and 3718 pixels of the pixelation area as clearly seen in figure (4B) of the histogram, which displays the arrangement of the peaks as blue, green and red with observed close space between blue and green, which may represent a certain type of lipid molecules, while the red colour

which is far from the two former colours may reflect other type of molecules in the lipid bimolecular layer.

In figure (7A) which represents the period of two hours after feeding, the lipid bimolecular layer is coloured with light yellow and in this figure, the protein layers are seen to be much intermingled with lipid layer. The histogram in figure (7B) give the arrangement of the peaks as blue, green and red with a value of 165.92 and the pixelation area is 6037 pixels, and it also reflects a great changeable in the arrangement of the peaks as clearly notice in the blue colour which locates at the beginning of X axis of the histogram while the red colour locates at the end of the axis followed inwards by the green one in a close distance. These features may interpret the changeable arrangement of molecules of plasma membrane and porosome during cell secretion.

In figure(5A), the secretory materials (the enzymes) appear in the period of one hour post- feeding as a deep green colour in the base of the flask-shaped porosome, in addition, some of them are seen in the lumen of the exocrine pancreatic acinus. They are marked in white dots and have a value of 49.86 and pixelation area is 3489 pixels as obviously shown in figure (5B) of histogram. The three colours; blue, red and green in arrangement of the peaks are presented in this histogram.

After two hours post-feeding period, the secretory materials (the enzymes) are seen in light green colour at the lumen only while the porosome is empty as obviously demonstrate in figure (8A). they have value of 92.46 and 2632 pixels of the pixelation area as reveal in the histogram of figure(8B) ,and the arrangement of the peaks of this histogram are red, blue and green.

An interesting observation is seen in the histograms of figures (5B and 8B) of the secretory materials, that the blue colour occur as a single line in concentration. It is in arrangement before the red in the histogram of one hour post-feeding while it came after the red in the histogram of two hours post-feeding, so, it can be neglected in both cases as compared with the layers of plasma membrane and porosome.

Also, the red and green colours in these histograms are not at the same arrangement, i.e. the red peak became far away from the green peak in the histogram of released secretory materials which are observed in the lumen. These two colours might represent the types of active enzymes in the lumen.

In the present study, the changeable features that appear in the coloured electron micrograph and histogram values of two hours post-feeding period reveal obviously that the plasma membrane and porosome are in dynamics and functional reconstitution as described in 2009, by Jena in his interesting two reviews, about the secretory portal in cells and the functional organization of the porosome complex and associated structures facilitating cellular secretion. Furthermore, Jena elucidated in these reviews that a specialized and sophisticated secretory machinery is developed even in single – cell organisms such as the secretion apparatus of *Toxoplasma gondii*, the contractile vacuoles in paramecium, or the various types of secretory structures in bacteria. Therefore, he found in not surprising that the mammalian cells such as human platelets have evolved such highly sophisticated and specialized cup-shaped supramolecular lipoprotein structures – the porosome complexes and porosome – like “canaliculi system” for the precise and regulated docking, fusion, and release of intravesicular contents from cells. So, it established that the porosome is a universal secretory machinery at the cell plasma membrane and it has various forms of specialized structures starting in nature from the single – cell organisms till the highly mammalian cells structure in the different organs of the body as clearly elucidated also by Jeremic, 2008; Cho *et al.,* 2009 and 2010; Trikha, *et al.,* 2010; Wheatley, 2010.

Beside these great and elegant efforts of these pioneer physiologist investigators in identifying porosome in different organs, the author of the present study as a cytologist utilized the computer-assisted morphometry to analyze quantitatively the shape and fine structure of plasma membrane and porosome in pancreatic acinar cell of post-feeding rat to present another method of evaluation of the dynamics, composition, and functional reconstitution of porosome and plasma membrane structures in coloured histograms and numerical values of the electron micrographs. Specially that the image processing and the numerical analysis approaches are applied in medicine and biology (Szilágyi, 2006; Sadaphal *et al.,* 2008). Beside, a plenty of image processing searches and articles have been released in a very wide scale of different science branches in 2011 from “IEEE transactions on image processing [TA]”.

Therefore, it found worthy that utilization and application of the image processing and numerical analysis techniques in the present study to evaluate and estimate the ultrastructural components of the plasma membrane and porosome of the pancreatic acinar cell, may can be also applied in cell and molecular biology branches in general.

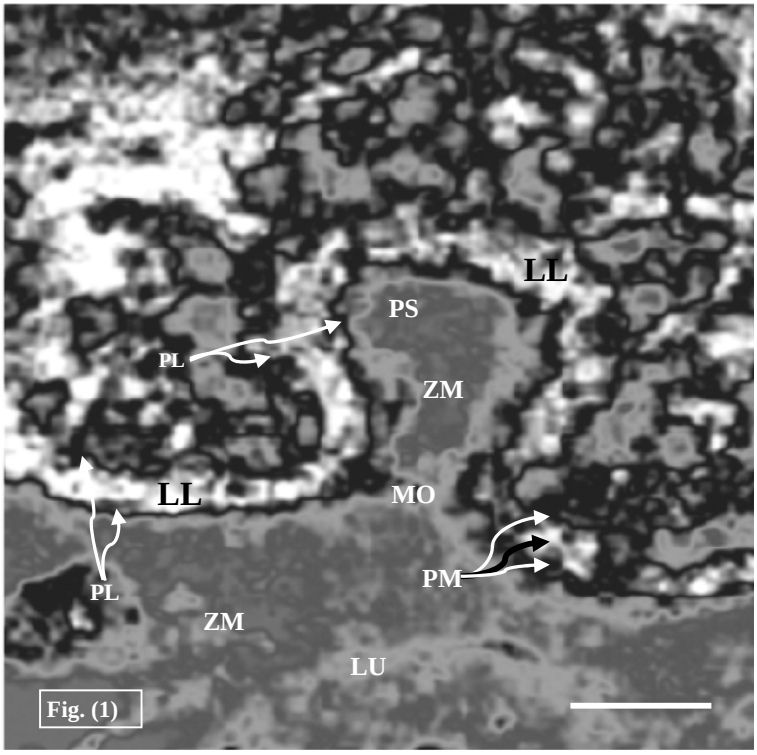


Figure (1): Electron micrograph of the apical region of a pancreatic acinar cell at one hour post-feeding period revealing the flask – shaped porosome **(PS)** with mouth opening **(MO)** range in size from 130 – 150 nm. It is loaded with electron dense particles representing enzyme materials **(ZM)**. Some of these particles are also seen at the acinar lumen **(LU)**. the plasma membrane **(PM)** showing the "tripartite structure" consisting of two dense bands representing protein layers **(PL)** separated by a central clear zone of lipid **(LL)**. (Scale Bar = 300 nm)

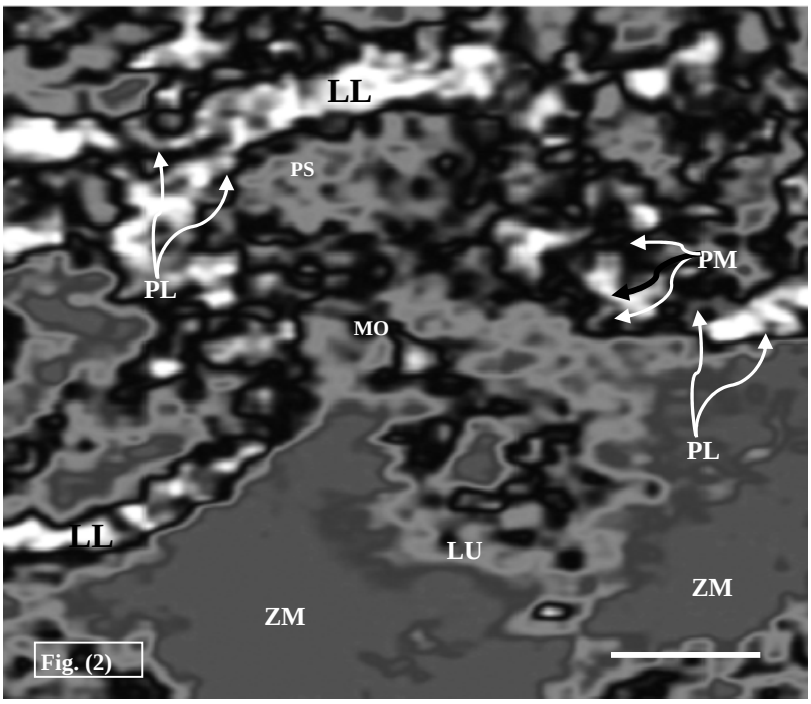
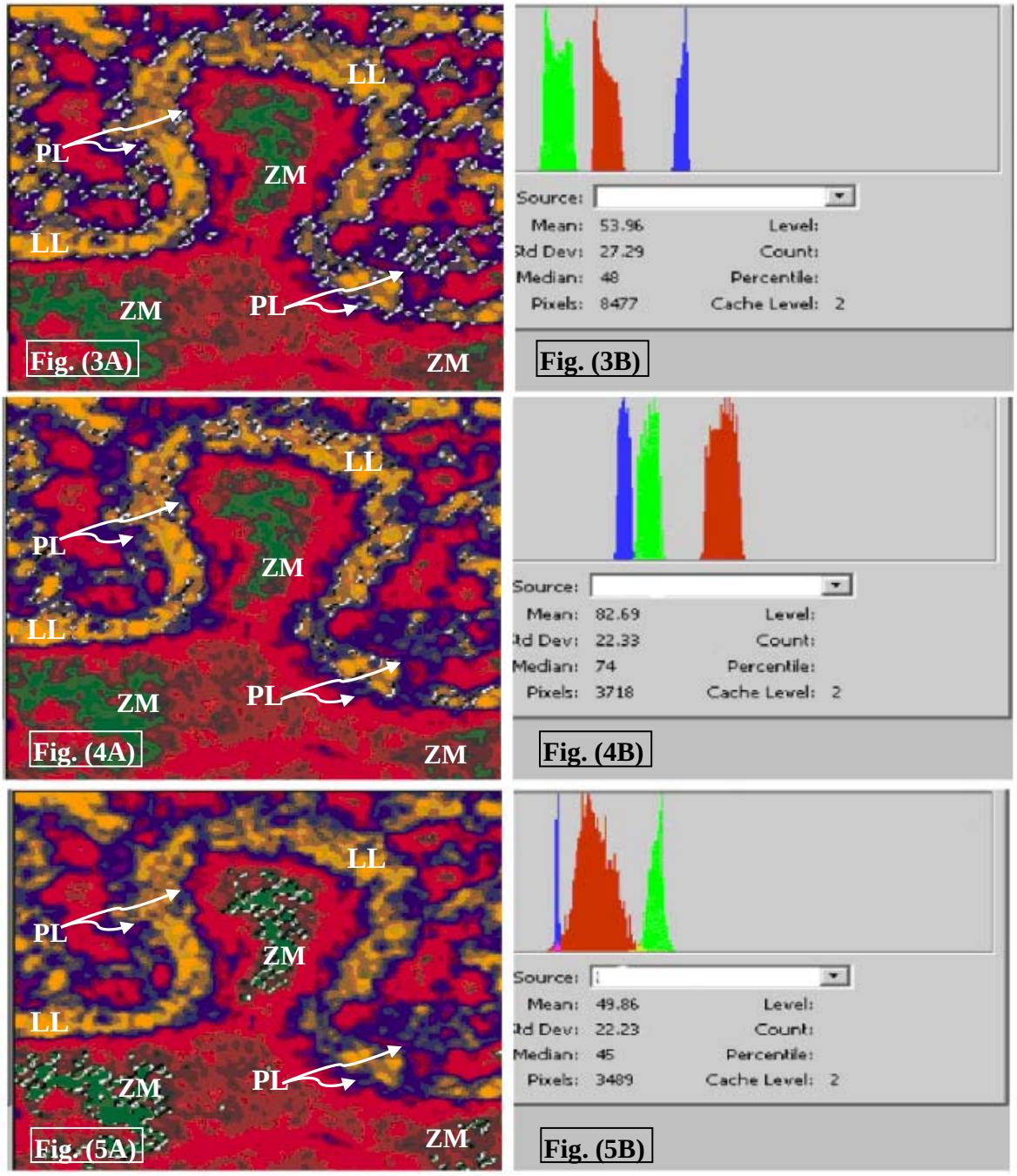
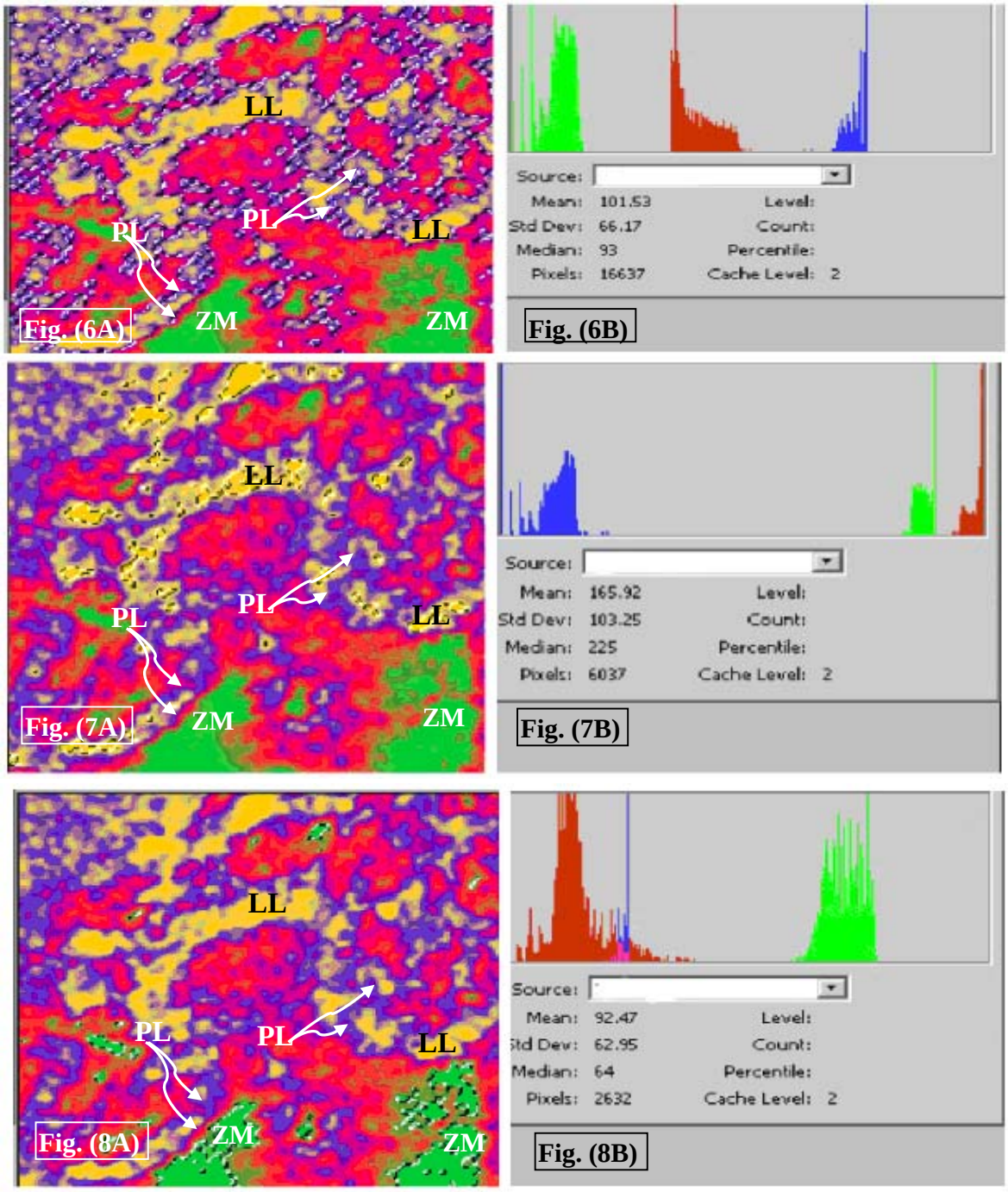


Figure (2): Electron micrograph of the apical region of a pancreatic acinar cell at two hours post-feeding period illustrating clearly cup-shaped porosome **(PS)** with a wide opened mouth **(MO)** (275– 300 nm) compared with figure (1). No dense particles can be detected at the groove of its cup, while the acinar lumen **(LU)** is loaded with these dense particles (enzyme materials) **(ZM)**. (Scale Bar= 300 nm).



Figures (3A, 4A, 5A) : Image analysis of the micrograph of one hour post-feeding period revealing obviously the tripartite structure of the plasma membrane and the porosome; an outer and an inner protein layers **(PL)** appear with blue colour, while the lipid bilayer **(LL)** that sandwiched between them appears with a variant degree of deep yellow colour which reflecting the presence of the molecules of its bilayer. The flask-shaped porosome has the same colour of plasma membrane unit on its three lateral boundaries. The enzymatic materials **(ZM)** in porosome groove and in lumen appear with a distinct colour of deep green.

Figures (3B, 4B, 5B) : Illustrating the histograms analysis of colours and the numerical values of protein layers, lipid layer and enzymatic materials respectively at one hour post-feeding period.
Figure (3B) : The protein layers arrangement of the peaks as green, red, blue with a value of 53.96 and 8477 pixels.
Figure (4B) : The lipid layer arrangement of the peaks as blue, green, red with a value of 82.69 and 3718 pixels.
Figure (5B) : The enzymatic materials arrangement of the peaks as blue, red, green with a value 49.86. and 3489 pixels.



Figures (6A, 7A, 8A): Representing image analysis of the micrograph of two hours post-feeding period. The plasma membrane unit and the porosome appear with the same colours as in the figures of one hour post-feeding period, but they have different arrangement on their molecular structures, while the enzymatic materials which is occupying only the acinar lumen appear with light green colour.

Figures (6B, 7B, 8B): Showing the histograms and the numerical values of protein layers, lipid bilayer and enzymatic materials respectively of the two hours post-feeding period. It is obviously noticed in these figures that although the plasma membrane unit and the porosome have the same colouration arrangement of their peaks as appeared in the histograms of one hour post-feeding period, yet they have different values in number according to their position that appear on the X axis as in the following:

Figure (6B): The protein layers in value of 101.53 and 16637 pixels.

Figure (7B): The lipid bilayer in value of 165.92 and 6037 pixels.

Figure (8B): The enzymatic materials in the lumen in value of 92.47 and 2632 pixels. (The position of the blue line can be neglected)

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