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TERMINOLOGICA

Concerning the concept 'organelle'

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Summary. Since the essential cytological functions are endergonic processes, the organelle cannot be defined without considering the necessary energy transfer. Therefore, a sensible definition may read: The organelle is a cytoplasmic structure with functional energy consumption.

The vital functions of ultrastructural cell differentiations consist of *endergonic* processes. Therefore, a definition of the organelle must include the capacity of energy transfer of the structure under consideration.

As a consequence, individual ribosomes are not organelles. Their function as synthesists of polypeptides is only possible in cooperation with a ribonucleic acid strand when they are organized as polysomes capable of putting into action free energy furnished by the surrounding groundplasm. So the polysome, the ATP converting system, must be interpreted as an organelle, although it is not enveloped by a biomembrane.

In the same way, an individual microtubule is not an organelle. Only an association of numerous microtubules cooperating with the energy furnishing groundplasm can function as an organelle (nuclear spindle, cortical microtubular system, etc.).

The same is true of individual enzyme molecules. Yet even crowds of such molecules suspended in the groundplasm or the enchylema[1] have not the status of organelles, in spite of their functional possibilities, because the catabolic breakdown which they catalize (digestion, fermentation, hydrolysis) are *exergonic* processes. Relevant experiments in vitro proceed without the addition of an energy donor (ATP, UTP, etc.) necessary for the functioning of the polysomal anabolism in vitro.

The structure which functions as an organelle in the case of hydrolases is the lysosome[2]. The lysosomal vesicles survive only as long as the surrounding groundplasm furnishes ATP or another donor of free energy. If this energy transfer is cut off, the enzymes in the vesicle not only hydrolyse its metabolites, but also autolyze the lysosomal membrane, previously maintained in a labile structural equilibrium, by a constant influx of energy.

Similar aspects are valid for the plasmalemma (plasma membrane). It can only resorb ions or molecules against their concentration gradients as long as it is furnished with free energy by the groundplasm.

Also groups of plasmic[3] or muscular fibrils which are involved in the dynamics of motility can only act as organelles if the necessary energy transfer is ensured.

In contrast to such open systems, the organelle status of coated vesicles with endergonic energy imput can more easily be demonstrated. This is true for the endoplasmic reticulum (synthesis of protein, intracellular nutritional transfer) or the Golgi apparatus (polymerization of oligosaccharides, secretion and excretion activity).

Thus the organelle can be defined as a cytoplasmic structural system which functions by endergonic energy transformation.

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PRO EXPERIMENTIS

Polyvinylchloride (PVC) particles implantation in mouse liver. A technique for experimental study of schistosome eggs-induced liver pathology[1]

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Summary. An injection of suspended PVC particles in the caecal vein of mice induces a foreign-body portal granuloma reaction in the liver. Plastic casts of the portal system, after PVC particles implantation, show modifications in the portal bed and are compared with plastic casts obtained in mice infested by *Schistosoma mansoni.* This technique can be useful to study the cellular dynamics of the portal granuloma and can be a model for schistosomal eggs induced liver pathology.

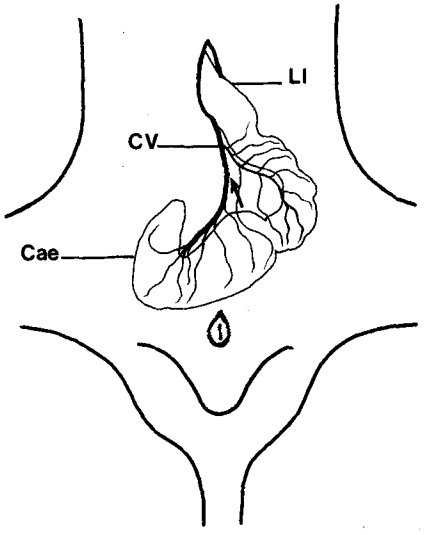

Fig. 1. Caecum position for PVC injection. Cae, caecum; CV, caecal vein; LI, large intestine. Arrow: point of PVC injection.

One of the most important factors in the pathological data of hepatosplenic Schistosomiasis Mansoni consists in granulomatous inflammation around the schistosome eggs trapped in the liver tissue[2]. Both the mechanical consequences of eggs deposit and the developing periovular granulomatous inflammation are responsible for extensive damage to the hepatoportal vascular system[3]. It is known that, in the initial stages of eggs production, there is relatively little host response, consisting mainly in a foreign-body reaction around the eggs. During this phase, the microvascular system of the liver is modified and a diffuse, nonspecific Kupffer cells response occurs. This initial stage is soon followed by a massive invasion of inflammatory cells corresponding to an immune response to soluble antigens secreted by the eggs[4].

In the absence of any specific immune stimulus, the intrahepatic PVC particles implantation in mouse liver was made to create an experimental model which would be useful to study, on the one hand, the cellular changes of the portal tract produced by foreign-body granuloma, and, on the other hand, the consequent hepatic vascular bed modifications.

Materials and methods. Swiss Albino mice were used: 5 control mice, 10 mice injected with polyvinylchloride (PVC) particles and 5 mice infested with 125 cercariae of Porto Rico strain of *S. mansoni*. They were sacrificed either 8 weeks after cercarial exposure or 8 days after particles injections.

Preparation of particule suspension: The PVC particles (Rhone - Poulenc - Polymere, Courbevoie, France) (110 ± 30 µm of diameter and 1.4 of density) are suspended in a polyvinylpyrrolidone solution before use.

Particles implantation: Mice (20–25 g) are anesthesized by parenteral injection of 0.04 ml of 5% sodium pentobarbital solution. A median longitudinal incision (2 cm) is made and the peritoneal cavity is opened. The caecum is taken out in order to expose the caecal branch of the mesenteric vein (figure 1). The caecal vein shows a linear portion about 2 cm long, where a particles injection can be done. 2 threads are placed under the caecal vein and 2 knots are prepared. The particles suspension is taken in a 1-ml syringe with 40 mm 8/10 needle and is introduced between the 2 knots in the direction of the liver. The proximal knot is tightened on the needle and the particles suspension (0.20 ml i.e. about 20,000 particles) is slowly injected (20 sec). The needle is removed and the knots are tightened. The caecum is put back into the peritoneal cavity and the abdomen is reclosed.

Light microscopy: The conventional technique after Bouin fixation, paraffin embedding and razor cutting was used.

Electron microscopy: The liver tissue is fixed in osmic acid (1%) buffered in sodium cacodylate (0.3 M) at 4 °C for 60 min). Dehydratation was carried out in ethanol and embedding was done in Epoxy-resin. Ultrathin sections for electron microscopy were cut, using an LKB Ultrotome III microtome, stained with uranyl-acetate/lead citrate solution, and observed on Philips EM 300 microscope. Semi-

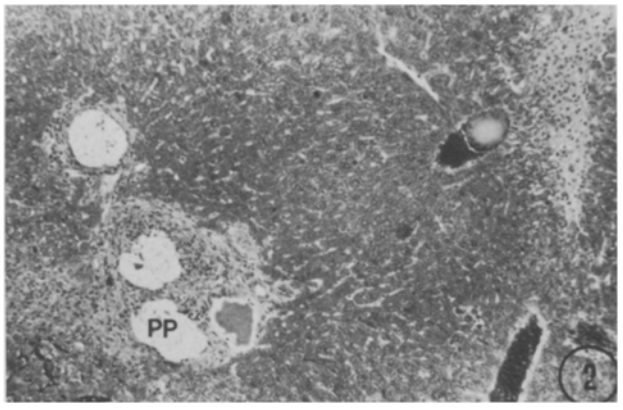

Fig. 2. Granulomatous reaction around 3 PVC particles. Light microscopic aspect. PP = PVC particle. × 63.

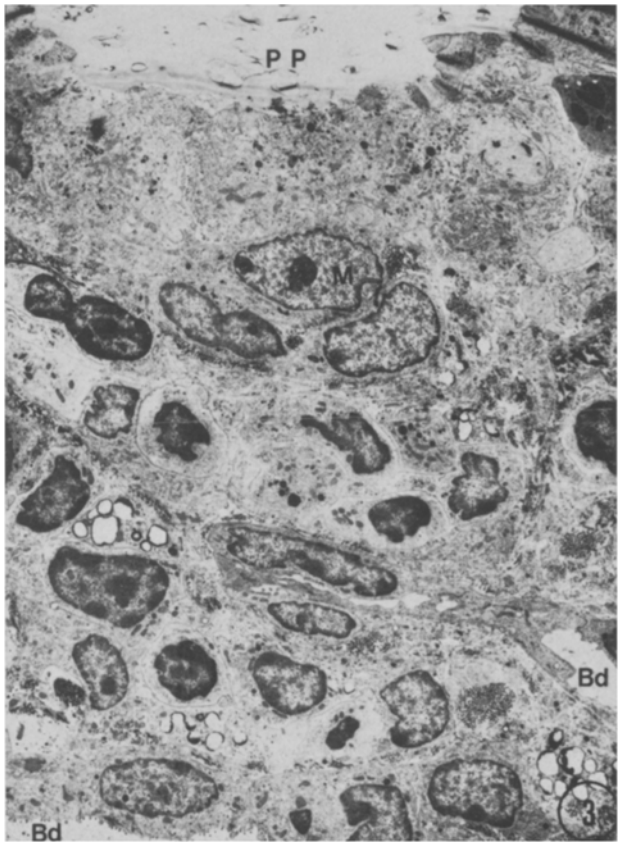

Fig. 3. Electron micrograph of a PVC granuloma. PP, PVC particle; M, macrophage; Bd, bile duct. × 2200.

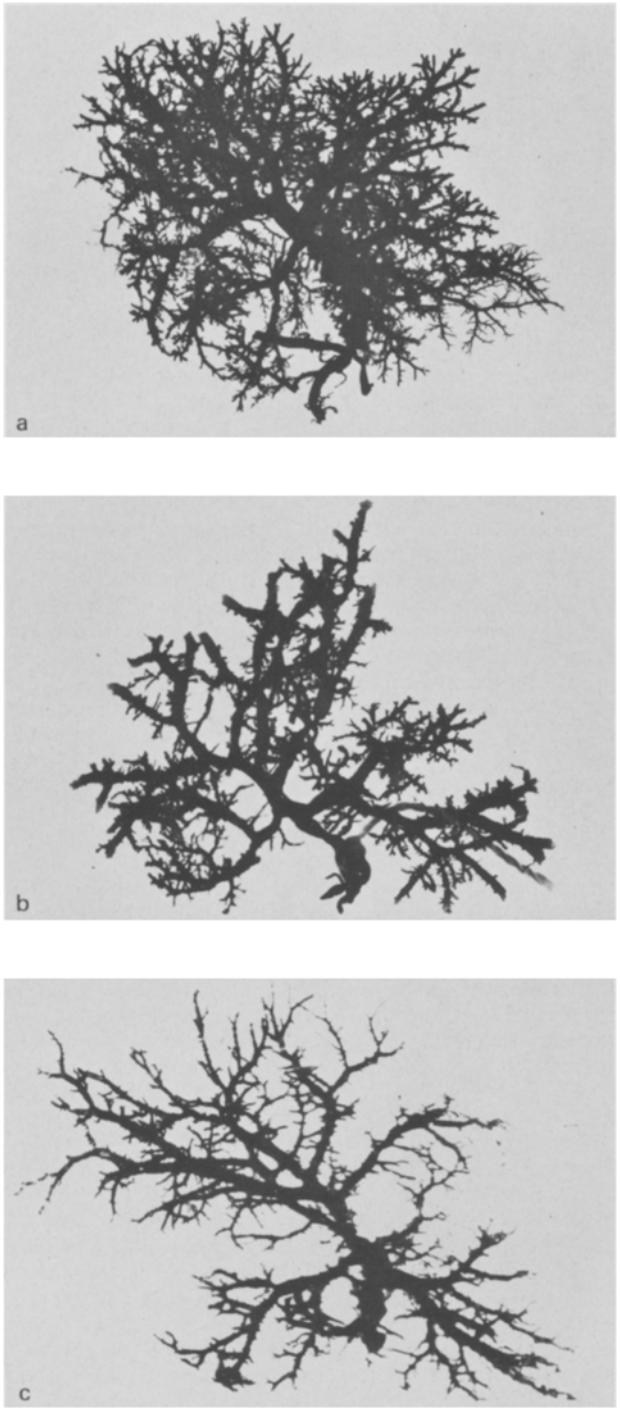

Fig. 4. Portal system casts of *a*, normal liver; *b*, schistosomal liver; *c*, PVC liver.

thin sections were treated following Richardson's technique to choose the blocks containing the particles.

Plastic casts of the portal system: A perfusion of vinylite (12% of vinyl chloride-acetate in acetone (Union Carbide Plastics CO. GRADE VYHH)) was done through the portal vein. After 12–24 h at 4 °C, the liver tissue was digested in concentrated chloridric acid for 6 h.

Results. The microgranulomatous aspect of the liver capsule shows a large and diffuse distribution of PVC particles through the hepatic parenchyma. On liver serial sections, light microscopy shows an homogenous repartition of PVC particles located in the interlobular veins, where they can be found either isolated or associated in small groups (2–5 particles). A granulomatous reaction is observed around each particle (figure 2) and a quantitative approach of inflammatory cells infiltration can be done. Electron microscopic examination of peri-particular granulomatous reaction (figure 3) shows numerous macrophages and giant cells in contact with the irregular surface particle. A lymphoplasmocytic, granulocytic and fibroblastic infiltration is associated. Plastic casts of the portal system show a significant decrease in the number of small injected veins, as compared to the controls (figure 4, a and b). A sudden reduction in the diameter of the medium and the small portal veins is observed. Narrow and dilated parts alternate with one another on several branches of the portal vein (figure 4, b).

In our series of PVC particles-injected mice, the reliability of the technique is greater than 50%. Unsuccessful results are due to post-operative mortality or to irregular distribution of PVC particles. Decrease in the number of small veins, and sudden diameter reduction or medium portal vein amputation are also observed in Schistosomiasis infected mouse liver (figure 4, c).

Discussion. Experimental studies of schistosome eggs-induced tissular pathology have been realized in lung by injecting eggs or inert particles through the tail vein of the mouse[4–7]. No report has been made on a specific technique of particles implantation in the liver.

Marked changes in the intrahepatic portal system are noted in human and murine hepatosplenic Schistosomiasis[8]. The reduction of the portal bed is a consequence of occluded portal branches by eggs and granulomas[3]. In our model this phenomenon is observed; the size and the localization of PVC particles associated with foreign-body granuloma lead to similar changes. In the absence of any specific host response, the present technique seems useful to test the consequences of permanent portal foreign-body modification on fibroblast stimulation, collagen deposit and fibrous tissue remodelling.

Such irritative permanent portal modification leads after a while to a new type of lobular vascularisation which probably includes a decrease of portal flow as well as an increase of hepatic arterial blood supply in the partial ligature of the portal vein of the mouse. This phenomenon is known as arterialisation of the liver in advanced human hepatosplenic Schistosomiasis[3].

A study of blood-hepatocyte barrier (Disse's space pathology) using this technique can be useful to identify non-specific chronic lobular modifications[9]. On the other hand, the coating of the PVC particles with labelled schistosome-egg antigenic fractions allow, through the electron microscope, the study of the cellular granuloma reactivity and composition[10,11].

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