C. Molecular chaperones in RER intracisternal crystals:

The formation of crystals in the RER intracisternal space of pancreatic acinar cells was induced by an intraperitoneal injection of DL-p-chlorophenylalanine methyl ester (CPME) into the animals. These features have been described by other workers (Arias and Bendayan, 1993; Forssmann and Bieger, 1973). Needle-like crystals with an electron opacity similar to that of the secretory zymogen granules, were found in the cisternal space of the RER (Fig. 1). The ribosomes at the RER membrane surrounding the crystals were much less numerous than those in the rest of the RER. The crystals presented a high immunolabeling for secretory proteins. Gold particles revealing amylase antigenic sites were primarily located over the intracisternal crystals as well as over all the cellular compartments along the secretory pathway (Fig. 2).

Immunocytochemical labeling for the cpn10, cpn60 and hsp70 confirmed our previous results (Velez-Granell et al., 1994), in which we demonstrated the presence of the three chaperones over the mitochondria and the different cellular compartments of the acinar cell secretory pathway. Both chaperonins, the cpn10 and cpn60, were located in the RER, Golgi apparatus, condensing vacuoles, and secretory zymogen granules, while hsp70 was mainly found over the RER and the trans-most Golgi cisternae (Velez-Granell et al., 1994). After the injection of CPME both cpn10 and cpn60 were found to be highly concentrated in the intracisternal crystals of the RER, while the hsp70 was not detected there. In fact
gold particles revealed the presence of cpn10 and cpn60 antigenic sites over the CPME-induced crystals as well as over the cellular compartments involved in secretion (rough endoplasmic reticulum, the Golgi apparatus, the condensing vacuoles and the zymogen granules) (Figs. 3,4). For the hsp70, gold particles revealing the presence of its antigenic sites were primary located over the trans-most cisternae of the Golgi apparatus in acinar cells from control and CPME-treated animals. In contrast to the cpn10 and cpn60, the anti-hsp70 antibody failed to demonstrate any specific signal over the intracisternal crystals (Fig. 5). The mitochondria of CPME-treated animals were less labeled than those from the control while gold particles were almost absent from nuclei and lysosomes. The pre-adsorption of each antibody with an excess of its corresponding antigen resulted in drastic reductions of the labeling, confirming the specificity of the antibodies. Similar results were obtained when the specific antibodies were omitted. These results confirmed the specificity of the labelings obtained.

Quantitative analysis of the labelings confirmed the morphological observations and demonstrated the increase along the secretory pathway of the cpn10 and cpn60 in control and CPME-treated animals (Fig. 6). The labeling densities in the crystals were 3 and 2 fold higher than those found over the zymogen granules for the cpn10 and cpn60, respectively (Fig. 6). Interestingly, labeling densities obtained for hsp70 contrasted with those obtained using anti-cpn10 or anti-cpn60 antibodies. Although the overall hsp70 labeling densities in CPME-treated animals
were about 50% lower than those of the control ones, no difference in the labeling pattern between normal and CPME-treated animals could be detected. In both cases, maximal hsp70 labeling was over the Golgi compartment (Fig. 6).
Figures and Legends

Figure 1: Electron micrograph showing features of pancreatic acinar cells displaying CPME-induced crystals. Crystalline electron dense inclusions (arrowheads) are located within the RER intracisternal space. The membrane surrounding the crystals is continuous with that of the RER (arrows). nucleus (N). mitochondria (M). zymogen granules (ZG). Magnification: 17000x.

Figure 2: Protein A-gold immunocytochemical detection of amylase, by pancreatic acinar cells displaying CPME-induced crystals. Gold particles revealing amylase antigenic sites are mainly located over the zymogen granules (ZG) and the intracisternal crystals (arrowheads). Golgi (G) apparatus. Magnification: 29000x.
Figure 3: Protein A-gold immunocytochemical detection of cpn10 antigenic sites on pancreatic tissue from CPME-treated rat. A large number of gold particles are located over the intracisternal crystals (arrowheads) and the zymogen granules (ZG). Some particles are also present over the Golgi area (G) and mitochondria (M). Acinar lumen (L). Magnification: 27000x.

Figure 4: Protein A-gold immunocytochemical detection of cpn60 antigenic sites on pancreatic tissue from CPME-treated rat. Intracisternal crystals (arrowheads) and zymogen granules (ZG) are highly labeled. Some labeling is also present over the mitochondria (M). Magnification: 26000x.
Figure 5: Protein A-gold immunocytochemical detection of hsp70 antigenic sites on pancreatic tissue from CPME-treated rat. The labeling is particularly concentrated in the Golgi area (G). Few gold particles are seen over the intracisternal crystals (arrowheads) and other cell compartments. zymogen granules (ZG). mitochondria (M). Magnification: 36000x.

Figure 6: Quantitative evaluation of labeling densities for the chaperonins and hsp70 proteins in acinar cells from normal (A) and CPME-treated (B) rats. Cellular compartments analyzed were: intracisternal crystals (CR), mitochondria (MIT), rough endoplasmic reticulum (RER), Golgi apparatus (GA), condensing vacuoles (CV), and zymogen granules (ZG). Values represent mean values ± SEM. N=15 or more. Comparative evaluation of the labeling densities using an ANOVA revealed significant differences (p<0.001) in all cases. Labelings for cpn10 and cpn60 but not hsp70 over the crystals were significatively different from those of the other cell compartments (p<0.001, Student unpaired t test).