GAP JUNCTION-RIBOSOME ASSOCIATION AFTER AUTOLYSIS

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Cell membrane integrity and regularity are criteria commonly used to appreciate the preservation of biological specimens in electron microscope preparations. Disruptions in the cell membrane are observed in tissues experimentally submitted to autolysis and are interpreted as an expression of the fragility of autolysed tissues during technical procedures. In liver cells, on which most of the studies on autolysis have been done (see review by Trump and Ericsson, 1965), the lesions of the nonspecialized portions of the cell membrane are described and interpreted as a key factor in the pathogenesis of the changes after autolysis (Trump et al., 1971). In the present work it is reported that the gap junctions are more resistant to experimental autolysis than the nonspecialized portions of the cell membrane, and it is demonstrated that there is an association between ribosomes and those junctions at different time intervals of autolysis.

MATERIALS AND METHOD

10 male rats, 3 months old, were utilized. The animals were sacrificed by decapitation and their bodies left at room temperature (22° ± 2°C) or placed in the refrigerator (4°C). At 3, 6, 9, 24, and 48 h after death, small pieces of liver were taken from both groups of animals. The following fixation and staining techniques were used: (*a*) 2% glutaraldehyde in phosphate buffer (Millonig, 1961) followed by 2% osmium tetroxide in the same buffer, sections stained with alcoholic uranyl acetate followed by lead citrate; (*b*) 2% glutaraldehyde in phosphate buffer (Millonig, 1961), sections stained with lead citrate; (*c*) 2% osmium tetroxide, sections stained with alcoholic uranyl acetate followed by lead citrate; (*d*) formaldehyde-glutaraldehyde fixative diluted 1:1 with 0.1 M cacodylate buffer (Graham and Karnovsky, 1966) to which 0.1% Alcian blue was added, postfixation in 1% osmium tetroxide with 1% lanthanum nitrate in *s*-collidine buffer (Shea, 1971), staining with uranyl acetate and lead citrate.

Thin sections were cut on an LKB ultrotome and observed in a Siemens Elmiskop 101 at 80 kV. 1 μm sections were made from the same blocks and observed in the light microscope after staining with toluidine blue.

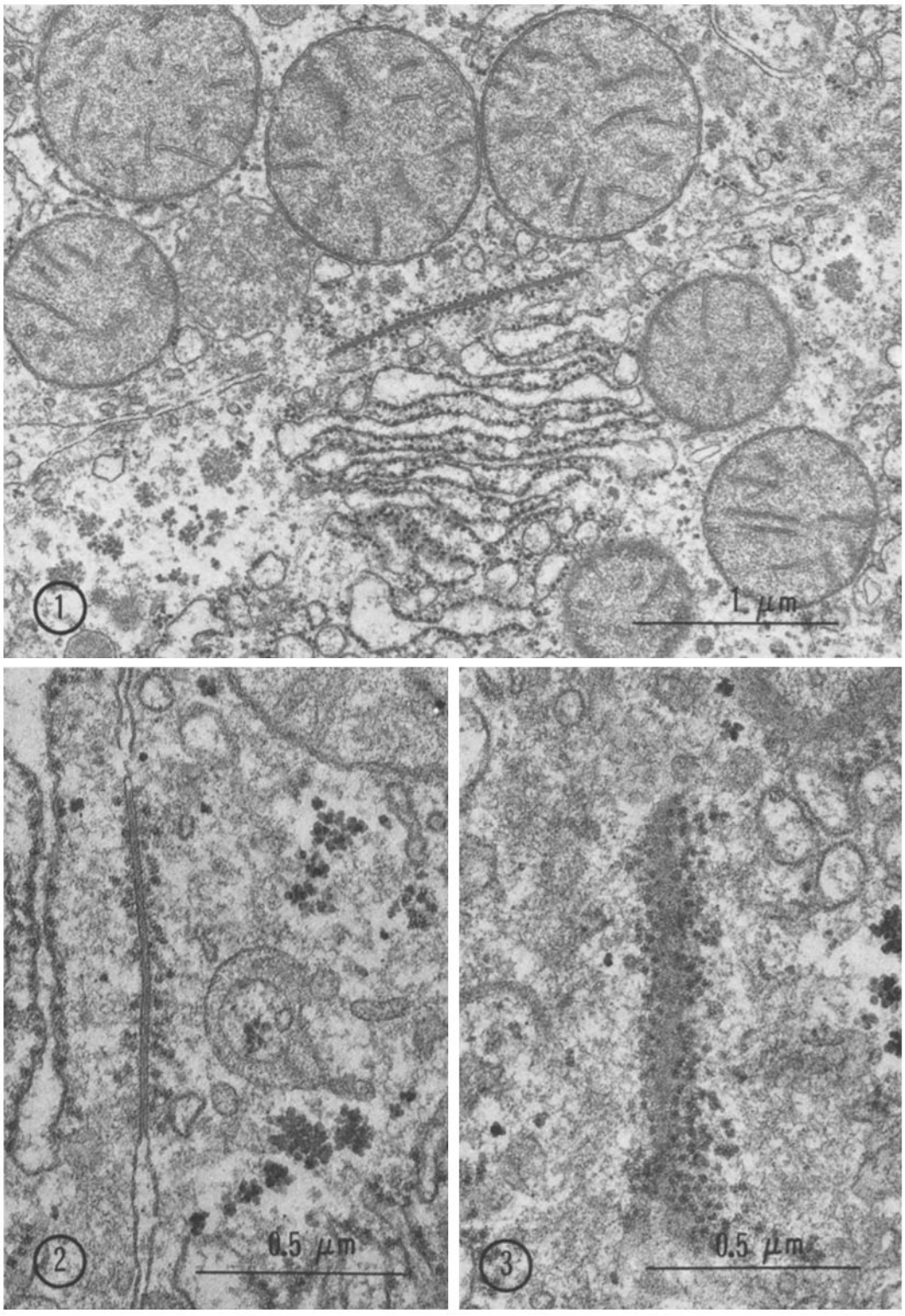
RESULTS AND DISCUSSION

The morphological changes observed in sections from specimens prepared during the autolysis experiments were essentially the same in both experimental conditions. However, for the same periods of time the tissues from the 4°C group were always better preserved than the ones from the group left at room temperature. The following cell alterations have been observed: *nuclei*—clumping of the nuclear chromatin, dark granules in the nucleoplasm, blebs in the outer membrane of the nuclear envelope; *mitochondria*—light matrix, disappearance of the dense gran-

FIGURE 1 Rat liver, 6 h of autolysis at room temperature (22°C). Double fixation with 2% glutaraldehyde and 2% osmium tetroxide. A low power view showing an area of apposition between two hepatocytes. The mitochondrial profiles are circular and the matrix is less dense than in the controls. Matrix granules are absent. ER cisternae are partially devoid of ribosomes. The membranes between the two cells show wide interruptions in their continuity but are intact in the portions correspondent to the gap junctions. Aligned on the cytoplasmic side of both membranes are dense structures 20–25 nm in diameter. × 32,000.

FIGURE 2 A higher magnification view of a gap junction from a specimen fixed 6 h after death (room temperature group). On the cytoplasmic side of both membranes forming the junction are more or less aligned structures with the same dimensions and density as the ribosomes attached to a nearby cisterna. × 64,000.

FIGURE 3 Tangential section through a gap junction from a specimen under similar experimental conditions as Fig. 2. Face view showing that the associated structures are granules with the same dimensions and density as the ribosomes. Some are forming small clusters. × 64,000.

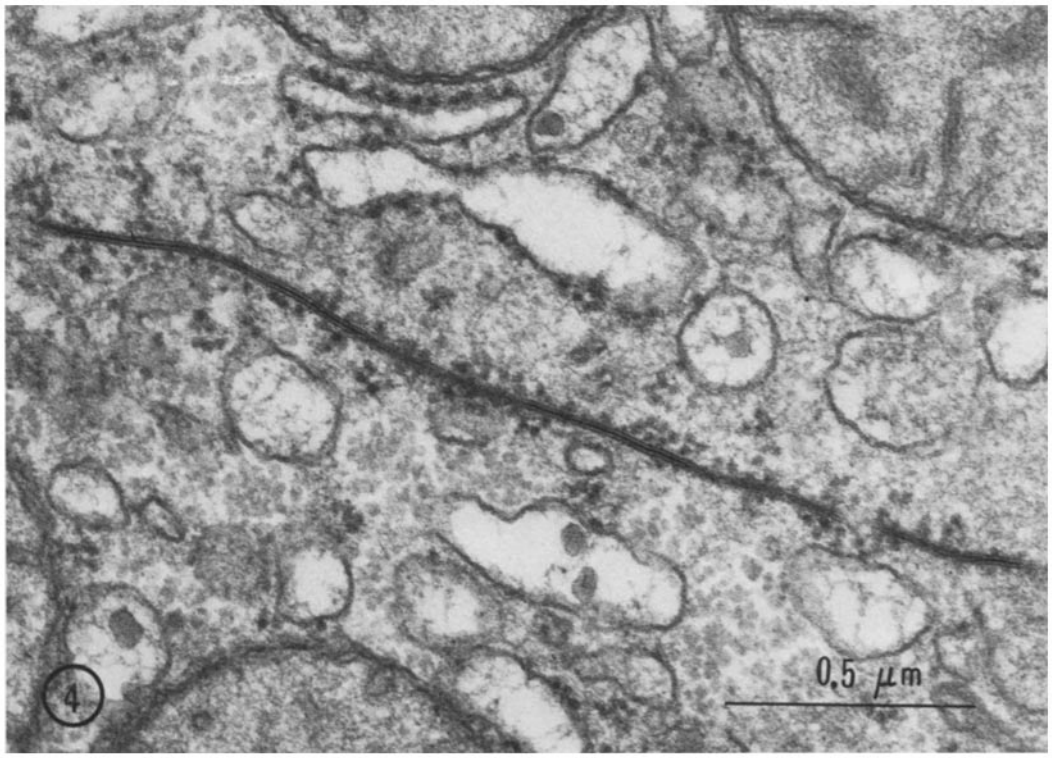


ules, cristae dilated and angular, giving place to myelin figures, disruption of the outer membrane; *rough and smooth endoplasmic reticulum (ER)*—the parallel arrangement of the cisternae is lost, ER-vesiculation and degranulation of the rough ER, presence of osmiophilic granules in their cavities and as autolysis progresses disappearance of the association of ER-mitochondria and ER-peroxisomes; *lysosomes*—intact limiting membrane up to late stages of our study, light matrix and presence of an increasing number of osmiophilic granules in their interior; *peroxisomes*—light matrix, preservation of the crystalloid and intact limiting membrane up to late stages. After 6 h of autolysis, are frequently observed areas of ground substance surrounded by a double or a single membrane (*autophagic vacuoles*). Those are the general alterations more frequently seen and already described in other studies on the same topic (Trump and Ericsson, 1965). The differences observed between the cold and room temperature groups were only in the extension of the lesions and in time of their appearance.

In respect to the cell *membrane* the more conspicuous alterations seen were membrane whorls associated with the membrane profile and interruptions in the membrane continuity. The size and number of these discontinuities increase with the duration of the experiment, but they appeared earlier in the tissues maintained at room temperature. In contrast with these lesions constantly observed in the nonspecialized zones of the membrane, some portions were never disrupted although neighboring membranes were seriously injured (Figs. 1 and 2). On the basis of their situation in relation to the bile canaliculi and their morphology, these zones were identified as gap junctions. This conclusion was confirmed by the observation of thin sections from specimens where lanthanum had been used as tracer (Fig. 4). In previous studies it has been demonstrated that the gap junctions are particularly resistant to experimental manipulations as in osmotic (Brightman and Reese, 1969), mechanical (Goodenough and Revel, 1970), and enzymatic disruption (Berry and Friend, 1969). From our observations it is concluded that those junctions are more resistant to autolysis than other zones of the cell membrane.

In tissues from specimens taken 3–9 h after death of the animal the presence of dense structures 20–25 nm in diameter associated with the gap junctions was a constant finding (Figs. 1–5). These structures were more or less regularly aligned on the cytoplasmic side of the membranes forming the junctions. This observation was made in tissues from both groups of animals and in the different fixing conditions used in this study. The structures were particularly numerous and evident in specimens taken at 6 h. Associations of the same type were never observed in relation with other portions of the membrane profile or in the controls. The possibility that the dense 20–25 nm structures represent cross sections of filaments running perpendicular to the plane of the sections has been excluded after observation of tangential sections of the same regions (Fig. 3). The dense structures are granules, apparently round, showing an irregular outline at higher magnifications. It was possible to recognize in some of the granules two asymmetric portions, and in some images they were observed to form small clusters (Fig. 3). By their stainability those granules look similar to the ribosomes seen in the neighboring cytoplasm, free or associated with the ER. The application of cytochemical procedures in order to reinforce that conclusion proved to be difficult because of the fragility of the autolysed tissues during the necessary technical manipulations. We have obtained additional evidence in sections from specimens fixed only with glutaraldehyde and observed after staining with lead citrate. In this case it was evident that the gap junction-associated granules stain like ribosomes (Fig. 5). Therefore, on the basis of their dimensions, morphology, and stainability, it was concluded that they were ribosomes attached to the gap junction membrane.

At this stage of our work we do not have a clear explanation for the observed ribosome-gap junction association. Not only is cell injury accompanied by many alterations which may be involved in the process but also the exact nature of the forces binding ribosomes to membranes is not yet fully understood. However, a tentative hypothesis can be assumed from the fact that during autolysis there is calcium influx into cells. From the work of Loewenstein (1966) it is known that cell uncoupling is one of the first reactions to cell injury, and it has been demonstrated (Loewenstein, 1966; Oliveira-Castro and Loewenstein, 1971) that the experimental introduction of calcium and other divalent cations into cells is followed by junctional sealing. This sealing has



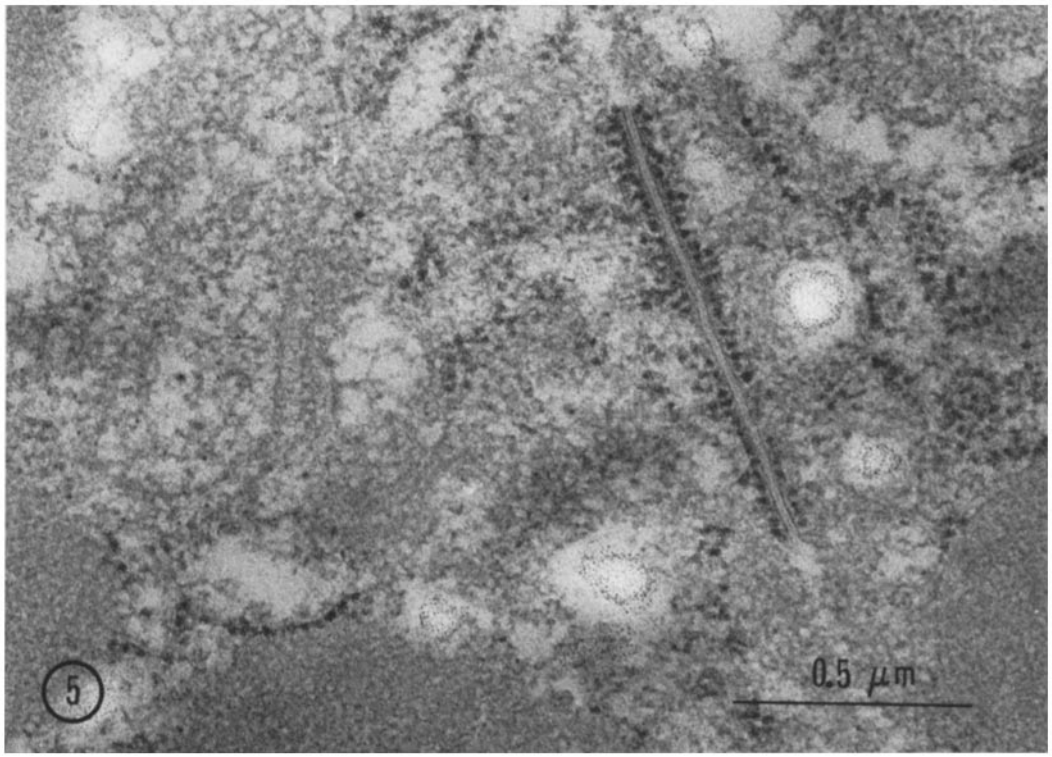


FIGURE 4 Autolysed rat liver cell. Alcian blue-lanthanum nitrate. A long gap junction with a small number of associated granules. × 64,000.

FIGURE 5 Rat liver, 6 h of autolysis at room temperature. Fixation in 2% glutaraldehyde; sections stained with lead citrate. The gap junction-associated granules are densely stained as are the ribosomes in the cytoplasm. × 64,000.

been explained by the binding of ions to the junctional membrane. To explain why during autolysis ribosomes are specifically associated with gap junctions, we can admit as a working hypothesis that ionic modifications occurring at the level of the junction are responsible for the binding process.

The ribosome-gap junction association demonstrated in the present work offers a new situation where the mechanism of ribosome binding to membranes can be studied.

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