Rickettsial infection in marine crustacea

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Summary. A rickettsial disease is described in the crab *Carcinus mediterraneus* Czerniavski. The pathogen develops within vacuoles in connective tissue cells. 2 morphologically different forms are observed in the last stage of infection. The systematic position of this procaryote is discussed.

Rickettsial diseases have recently been described in marine molluscs[2–5], but they are not known in marine crustacea even though they have been observed in terrestrial and freshwater species[6,7]. In the course of an ecopathological study of the crab *Carcinus mediterraneus* CZ., besides a *Baculovirus*[8] causing deaths, rickettsia-like organisms were observed in the connective tissue between the hepatopancreatic tubules of diseased crabs not affected by the virus. We studied the structure and the pathogenic effect of these germs.

Methods. Infections were experimentally obtained by inoculating healthy crabs with a 0.2 ml suspension of diseased hepatopancreatic tissue in ultrafiltered and sterilized water. For histological studies, tissues were fixed in Davidson solution [9], and sections stained by Feulgen's, Mann-Dominici's and Gram's methods[10,11]. For ultrastructural studies, the material was fixed in a 3% buffered glutaraldehyde-2% osmium tetroxide[12], and embedded in epon. Ultrathin sections were stained by the method of Reynold[13].

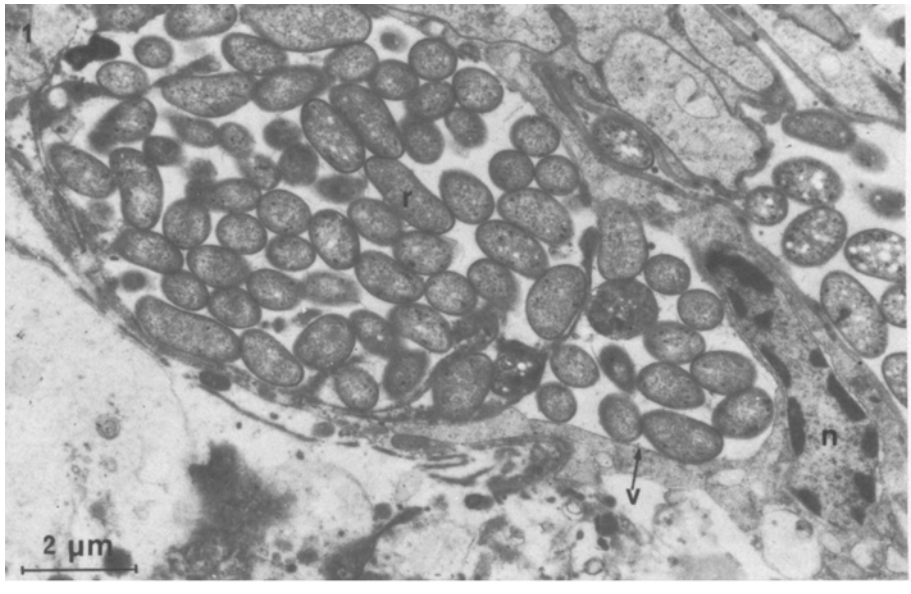

Fig. 1. Young microcolony of rickettsia within a connective tissue cell. v, vacuolar membrane; n, nucleus; r, vegetative stage of rickettsia.

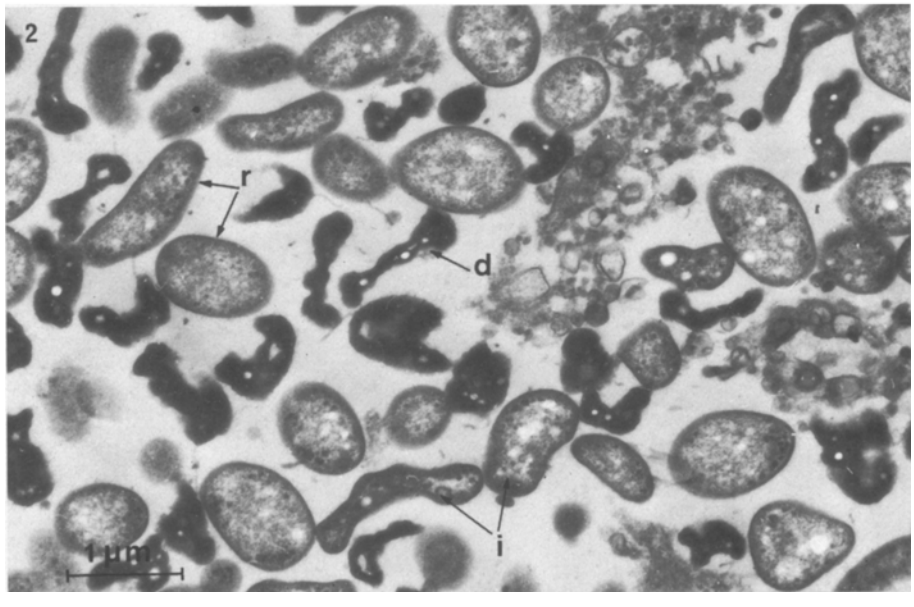

Fig. 2. Microcolony after the opening of the vacuole in the intercellular space. d, electron-dense bodies; r, vegetative stage, i, intermediate stage.

Results. Inoculated animals died 15 days after infection, showing the histological alterations peculiar to the rickettsial infection. The microorganisms are located in the cytoplasm of connective tissue cells in the hepatopancreas, gut, gills, and gonads. These gram-negative, rod-shaped procaryotic cells (2 μm × 0.7 μm), form Feulgen-positive microcolonies of 10-20 μm diameter. They are non-motile and uncultivable in the usual bacterial media.

Observations by electron microscopy revealed that the microorganisms multiply within intracytoplasmic vacuoles. They are bounded by a plasma membrane and a cell wall, both displaying the structure peculiar to the unit membrane, separated by a light area of about 150 Å thick. They contain many ribosomes and fibrils of a nuclear material. Some rod-shaped forms are transversely constricted, showing binary fission.

After these procaryotes are released by the rupture of the cell in the last stages of infection, ultrastructural changes occur: ribosomes are located at the periphery of the microorganism, which becomes electron-dense, and a cytoplasmic shrinkage involves a deformation of the rickettsia which exhibits an important pleomorphism.

Discussion. Considering its intracytoplasmic development and its ultrastructure, this pathogen is to be ranged among the wide group of Rickettsiales. The intravacuolar position and the presence of electron dense forms showing some characteristics of resistant bodies (besides degenerating forms), suggest that this rickettsia may be related to a group whose position is presently under consideration[14-17] and in which several *Wolbachia* and *Rickettsiella* are involved. Finally, it should be emphasised that this disease is experimentally reproducible and that this study is the first description of a rickettsial infection pathogenic to marine crustacea.

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Chromatin condensation in isolated rat hepatocyte nuclei induced by ribonuclease treatment[1]

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Summary. Ribonuclease treatment of isolated rat hepatocyte nuclei induced a 75% solubilization of hnRNA, a reduction of the quantity of perichromatin fibrils, and a marked condensation of chromatin.

Chromatin in eukaryotic cells assumes 2 morphological patterns: a dispersed and a condensed form. The dispersed chromatin contains a larger amount of polyanionic substances (acidic nuclear proteins, phosphoproteins, RNA) than the condensed form[2,3]. Recent results on the relationship between these polyanionic substances and chromatin ultrastructural pattern have demonstrated that chromatin morphology is not influenced by acidic nuclear proteins, nor by their phosphorylation[4]. Dispersal and condensation of chromatin have been observed to be related, respectively, to a high or a low quantity of associated perichromatin fibrils[4-6] which are the morphological substrate of newly synthesized heterogeneous (hn) RNA[7,8].

To investigate the significance of the hnRNA in determining the chromatin ultrastructural pattern, in the present experiments we considered the effect of ribonuclease treatment on chromatin morphology in isolated regenerating rat hepatocyte nuclei. The aim of this work was to demonstrate that the solubilization of hnRNA might induce a condensation of the dispersed extranucleolar chromatin, thus showing that the ultrastructural pattern of chromatin is related to the quantity of the transcriptional products associated with the DNA-histone complex.

Experimental. Rats of the Wistar strain, weighing 130-150 g, were used. 24 h after partial hepatectomy[9], liver RNA or DNA was labelled by i.p. injection of 6-[^{14}C]- orotic acid (sp. act. 57 mCi/mmole)[10] or methyl-[^{3}H]- thymidine (sp. act. 21 Ci/mmole)[10]. Livers were homogenized with 9 vol. of cold medium A (0.25 M sucrose, 3 mM $MgCl_2$, 2 mM K phosphate buffer pH 6.8). After centrifugation at 1200 g for 10 min at 4 °C, the pellet was washed once with medium A and suspended with 5 vol. of medium A. Samples, 0.5 mg DNA, were incubated in a medium containing 0.25 M sucrose, 50 mM Tris-HCl at pH 7.5, 1.4 mM K phosphate buffer at pH 6.8, 3 mM $MgCl_2$, in the presence or absence of 80 μg RNase (Sigma type IIS from bovine pancreas) previously incubated at 70 °C for 15 min. After the incubation at 20 °C for 10 min the preparations were kept in an ice water bath for 10 min. After this time, samples were taken and RNA, DNA and radioactivity were measured[10,11]; the remaining part was processed for the electron microscopic study[4]. Nuclear RNA was fractionated by treatment with phenol at different temperatures[12,13]. Control samples for biochemical analysis were processed before the incubation at 20 °C.

Results and discussion. We took care to establish that the ultrastructural pattern of control isolated nuclei was the same as that described in in-vivo generating hepatocytes at the corresponding time (24 h) after partial hepatectomy[5]. With the isolating procedure used, the morphology of