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.

1 This work was performed within the framework of a CNEXO-INRA agreement.
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Chromatin condensation in isolated rat hepatocyte nuclei induced by ribonuclease treatment[1]

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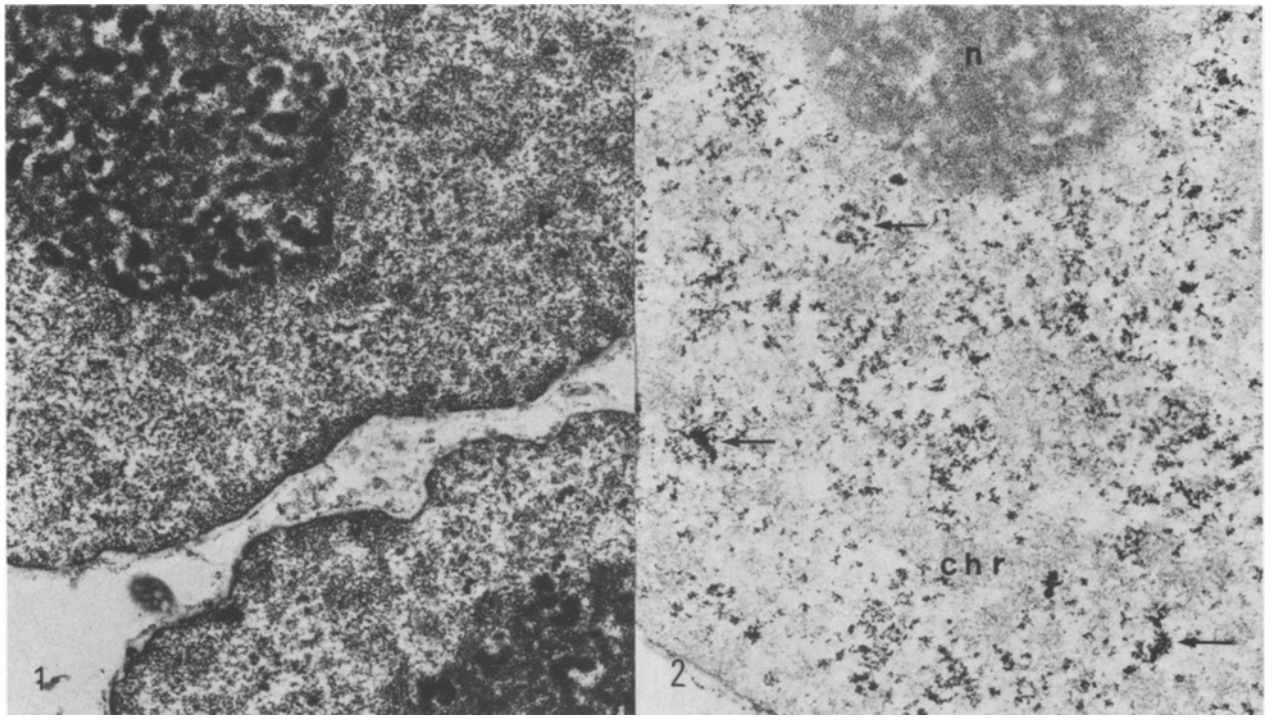
Summary. Ribonuclease treatment of isolated rat hepatocyte nuclei induced a 75% solubilization of hnRNA, a reduction of the quantity of perichromatin fibrils, and a marked condensation of chromatin.

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].

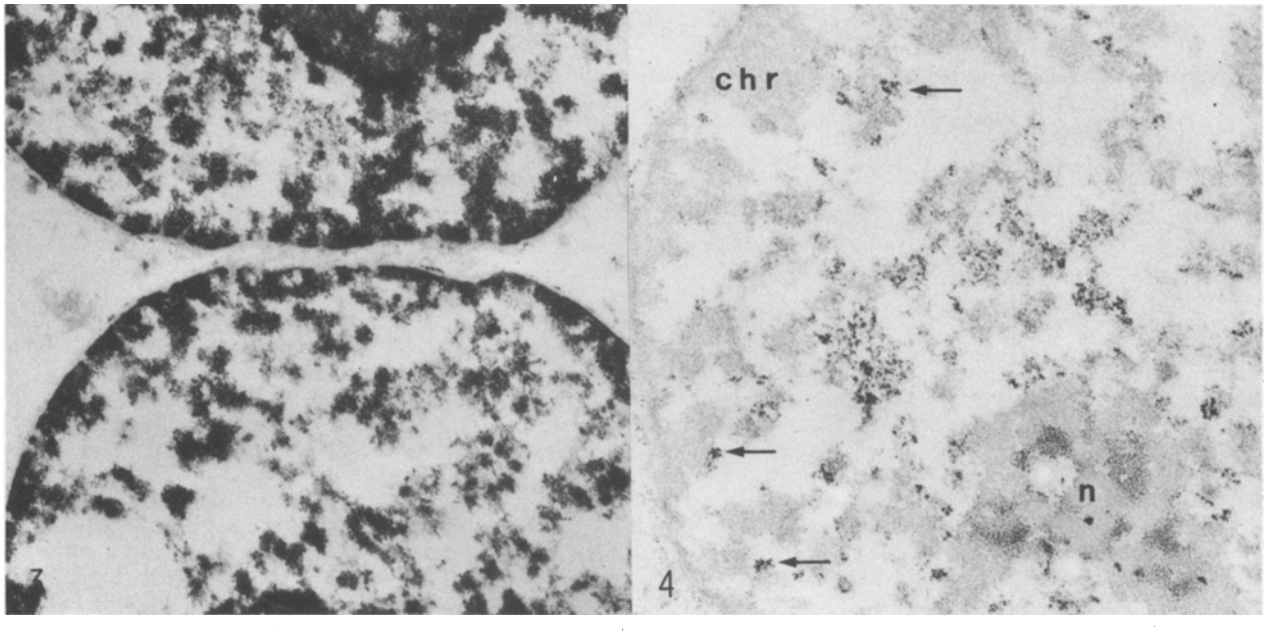
To investigate the significance of the hnRNA in determining the chromatin ultrastructural pattern, in the present experiments we considered the effect of ribonuclease treatment on chromatin morphology in isolated regenerating rat hepatocyte nuclei. The aim of this work was to demonstrate that the solubilization 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



Figures 1 and 2. Isolated hepatocyte nuclei from regenerating rat liver. Controls. Fig. 1. Almost all the chromatin is in a loosened pattern. In the large nucleoli the fibrillar and granular components are clearly visible. Uranyl and lead staining. × 16,300. Fig. 2. A large quantity of perichromatin fibrils (arrows) is visible throughout the nucleoplasm. In the nucleolus (n) the fibrillar and granular components normally intermingle. Chr, unstained chromatin. Uranyl-EDTA-lead staining. × 25,800.



Figures 3 and 4. Isolated hepatocyte nuclei from regenerating rat liver after ribonuclease digestion. Fig. 3. The chromatin appears condensed into electron opaque masses. The nucleoli look very compact. Uranyl and lead staining. × 16,300. Fig. 4. The quantity of perichromatin fibirls (arrows) is strongly reduced. In the nucleolus (n) the granular component is visible. Chr, unstained chromatin. Uranyl-EDTA-lead staining. × 25,800

nuclear components was the same as that described in vivo: nearly all the chromatin appeared in the dispersed form (figure 1) and the quantity of perichromatin fibrils (visualized with the Bernhard technique preferential for RNP[14]) was very high (figure 2). The treatment with ribonuclease for 10 min at 20 °C (the control nuclei were incubated in the same medium without enzyme) induced a clear condensation of the dispersed chromatin (figure 3) and a marked reduction of the quantity of perichromatin fibrils (figure 4). The ribonuclease treatment induced a 79% solubilization of ^{14}C-orotic acid, rapidly incorporated into total RNA. To evaluate the effect of RNase treatment on hnRNA, nuclear

Radioactivity of RNA and DNA after ribonuclease treatment.

Incubation	Ribonuclease 80 µg	Total RNA[a] dpm/µg DNA	Percent of control[c]	Phenol extracted RNA[a] 85 °C nuclear RNA fraction dpm/µg RNA	DNA[b] mg/incubated sample	dpm/mg DNA
none[c]	–[c]	99.73	–	123	0.542	43
10 min at 20 °C	–	88.20	88	120	0.540	42
10 min at 20 °C	+	20.76	21	31	0.542	42

[a] Animals were injected with 6-[^{14}C]orotic acid[10]. [b] Animals were injected with methyl-[^{3}H]thymidine[10]. [c] Sample was treated with 0.6 N PCA[11] without incubation and used as control.

RNA fractions were extracted by treatment with phenol at different temperatures[12,13]. The specific radioactivity (dpm/µg RNA) of 85 °C nuclear RNA fraction, that corresponds to hnRNA[12,13], is reported in the table. The reduction of the specific radioactivity to 25% of the control value demonstrated that the rapidly labelled hnRNA is degraded by the RNase treatment of the 1200 g nuclear pellet. Under the same experimental conditions no difference between PCA precipitated control and incubated samples was observed (table) regarding the DNA content and the specific activity (dpm/mg DNA) of thymidine incorporated into DNA.

The present findings show that the reduction of the quantity of perichromatin fibrils (hnRNA) induced a rapid condensation of extranucleolar chromatin and demonstrated that the condensed and dispersed forms of chromatin are due to a low or a high quantity of newly synthesized hnRNA respectively.

1 This investigation was supported by grants from C.N.R. (Roma) and by Pallotti's Legacy for Cancer Research.
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Tissue- and stage-specific RNA patterns in *Drosophila* as revealed by gradient formamide gel electrophoresis

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Summary. The in vitro labeled RNAs in the accessory glands from male adults, and in the salivary glands and fat body from developing larvae of *Drosophila melanogaster* were separated on linear gradient acrylamide gels in formamide. The patterns appear to be tissue- and stage-specific, and several lines of evidence indicate that the visualized radioactive bands include both hnRNA and messages.

We have previously shown that protein synthesis in the lethal mutations *1(3)tr* and *1(2)me* of *Drosophila melanogaster* is greatly reduced compared to that in the wild type at corresponding ages[2,3]. On the other hand, we found that the production of secretion proteins in the accessory glands (paragonia) of male adults is distinctly elevated following copulation[4]. In both cases it would be of interest to know whether corresponding alterations in the synthesis of messages in the fat body or paragonial cells take place. The study of RNAs in differentiated cells suffers from 2 handicaps: 1. In order to have sufficient homogeneous material for biochemical analysis each tissue has to be dissected out individually from the larva or fly. 2. Because of the extremely heterogeneous nuclear RNA (hnRNA) no satisfactory separation of the different RNA species can be achieved by conventional gel electrophoresis. In an effort to overcome these difficulties we have recently worked out a highly sensitive microelectrophoretic procedure by using formamide to eliminate the conformational effect of the RNA molecules[5], which are then separated on a linear gradient acrylamide gel.

Materials and methods. The wild type (Sevelen) of *Drosophila melanogaster* was cultured on a standard diet containing maize, agar, sugar and yeast at 25 °C. Accessory glands from male adults and salivary glands or fat body from larvae of the desired age were dissected out in a drop of cold Mops buffer solution and incubated for 60 min with tritiated uridine, cytidine, adenosine and guanosine (The Radiochemical Centre, Amersham; 6.4–52 Ci/mM, 10 µCi/µl). The in vitro labeled RNAs were extracted with 0.1 M acetate buffer (pH 5) and 80% phenol at 65 °C and precipitated by adding 2.5 volumes of ethanol. Following centrifugation the RNA precipitate was taken up in 98% formamide (Fluka) and heated at 100 °C for 4 min.

Stock solutions described previously by Mitchell et al.[6] were used for preparation of slab gels (0.18 × 10 × 11 cm). Monomer solutions containing 3.5% and 5% acrylamide in formamide were pumped between 2 glass plates with a