before dehydration in graded ethanols through propylene oxide into epon 812 or araldite. Some of the pieces were stained in block with uranyl acetate in pH 5 veronal acetate buffer. Sections were cut approximately 200-nm-thick and contrasted with uranyl acetate and lead citrate for examination.

Results. Scanning electron microscopy. The necrosis in the lung tissue was so advanced that it was only possible at low magnifications to identify the tissue at all as lung, and then only by recognition of a few remaining alveolar spaces and their interalveolar septa. Even these sparse alveoli were badly occluded with cells and debris. In them no type II epithelial cells were found but there were type I cells and a pletheora of other loose cells, many of which resembled macrophages but which could as well be polymorphonuclear leukocytes and lymphocytes active in the necrosis.

Small microorganisms were found extracellularly, usually lying on the free surfaces of the few recognizable alveoli, particularly in the more badly damaged regions of the tissue. They varied considerably in their size, shape and morphological appearance by SEM (figures 1–3). They were often found in the process of binary fission (figure 3). Some (figure 1) were relatively long and thin, with slightly tapered, sometimes almost pointed tips and uneven outlines. They were generally describable as 'rod-like' and frequently bore small surface bumps (diameters 0.06 μm), which we have reason to believe are not contaminant. Their surfaces were uneven and often bore low ridges or folds. Others, describable as 'sausage-shaped' (figure 2), were measurably shorter and plumper than the first, with well rounded sides and tips and smoother surfaces. These, too, had small bumps on their surfaces but no folds or ridges. If they are assumed to be the same organism as the first, the mean length and width for the combined total distribution of 71 of them were 1.84 and 0.56 μm, respectively, with a ratio of length to width of 3.3, in close agreement with measurements reported by others[5] from TEM of the microorganism.

Transmission electron microscopy. In thin sections (figures 4–6), the microorganisms possess a limiting cell envelope comprised of a triple membrane, 2 dark membranes separated by a clear space. The thickness of the triple membrane is 11–14 nm. The outer membrane was sometimes loose and undulating, in agreement with the SEM observations of surface folds or ridges. The cell contents are granular due to the presence of small particles (25 nm), the same size as ribosomes (figures 4–6). Fine thread-like structures (diameter 6 nm), were sometimes seen in their cytoplasm (figures 4 and 5), and many, but not all of the sectioned organisms demonstrated vacuoles within them, ranging from 30 to 160 nm in diameter (figure 6). Nuclear material was seen in some (figure 5), but not in all of them.

Discussion. The morphological characteristics of the microorganisms reported here from both SEM and TEM agree closely with those reported from TEM alone by others[5] for the responsible microorganism, described as the etiological factor in Legionnaires' disease. In addition, many other forms as well as a wider distribution of possible rod bodies have been sighted by SEM. A full discussion and description of these forms will be the subject of a later report. For example, in addition to the forms of microorganisms reported here, there is a great variety of rounded or oval bodies with a wide distribution of diameters. Some of these are very small (length and/or width approximately 0.1–0.3 μm) and representatives can be seen at the arrows in figure 3. Others can be quite large, as in figure 7, where the triple-membraned, rounded body is over 3 μm in diameter. These rounded bodies or spheroplasts resemble mycoplasmas[6] by TEM in thin section. However, in sections they do not seem to contain the small, ribosome-like particles which were characteristic of the bacterium-or rickettsia-like organism in thin section.

Efforts to detect the presence of the element nickel in the tissue and bacterium by both fluorescent X-ray and energy dispersive X-ray analysis were unrewarding and it was concluded that no nickel was present. This effort was stimulated by the suggestion put forth by Dr W. Sunderman, Jr at the time of the initial outbreak in Philadelphia. He claimed that nickel was present in the tissues of patients who died of Legionnaires disease.

1 This work has been supported in part by a Henry Ford Hospital institutional grant from the Ford Foundation and in part by the Veterans Administration Medical Center Research Funds.

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Reassociation of eukaryotic ribosomal subunits by a factor from rat ascites hepatoma cytosol

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Summary. Post-ribosomal supernatant extracts from Yoshida AH 130 ascites hepatoma cells promote the in vitro association of ribosomal subunits at low Mg^{2+} concentration. Comparable extracts from rat liver show, on the contrary, dissociation factor activity on ribosome monomers.

The dissociation of ribosome monomers into subparticles is a requisite for the initiation of protein synthesis[2]. Factors with that activity have been found in the cytosol and in the extract prepared by high salt wash from ribosomes in a number of eukaryotic cell types[3–8], including the mouse ascites cells[9] and the rat AH 130 Yoshida ascites hepatoma[10].

The in vitro activity of initiation factors appears to increase in transplantable hepatomas[11,12]. However, the in vivo sedimentation patterns of ribosomes obtained from these

Activity of the high salt ribosomal wash and post-ribosomal supernatant fraction preparations from rat liver and from Yoshida ascites hepatoma cells. Mean ± SE. The percent of dissociation (+) and association (−) of ribosomal subunits is also given. Numbers in brackets are number of experiments

Source of fraction	Controls	Ribosomal wash extracts		Supernatant extracts	
		100 µg protein	200 µg protein	100 µg protein	200 µg protein
Liver (12)	0.507 ± 0.0517	0.585 ± 0.0456 + 15%	0.678 ± 0.0393 + 34%	0.630 ± 0.0477 + 24%	0.691 ± 0.0535 + 36%
Ascites hepatoma (18)					
3 days after implantation	0.492 ± 0.0203	0.594 ± 0.0281 + 21%	0.642 ± 0.0236 + 30%	0.360 ± 0.0387 − 27%	0.290 ± 0.0240 − 41%
6 days after implantation	0.582 ± 0.0248	0.615 ± 0.0199 + 6%	0.675 ± 0.0667 + 16%	0.275 ± 0.0352 − 53%	0.120 ± 0.0290 − 79%
12 days after implantation	0.543 ± 0.0165	0.571 ± 0.0203 + 5%	0.602 ± 0.0185 + 11%	0.081 ± 0.0056 − 85%	0.021 ± 0.0036 − 96%

and other tumours, including the Yoshida ascites hepatoma[13–15], show abnormally elevated 80S monomer peaks in respect to normal liver. These observations might suggest the presence of a decreased activity of the ribosomal dissociation factor in tumour cells and a reduced ribosome recycling into active subunits in this condition[16].

In the present work, we have studied the activity of this factor in the high salt ribosomal wash and post-ribosomal supernatant (cytosol) extracts of Yoshida ascites hepatoma AH 130 cells at different periods of growth, as compared to normal rat liver. The results suggest the presence in the ascites hepatoma cytosol of a factor(s) which is able to induce the in vitro association of ribosomal subunits at low Mg^{2+} concentration.

Material and methods. Male Wistar rats weighing 180–250 g (2–3 months old) fed ad libitum until death and housed with fixed artificial illumination from 07.00 to 19.00 h were killed at the same day-period (09.30 h) by cervical dislocation and bled. The livers were rapidly extracted, weighed and homogenized at 0–4 °C as described[17]. Ribosomes and the post-ribosomal supernatant were obtained by conventional methods[10,12,17,18]. The ribosomal dissociation factor was obtained by high salt wash from ribosomes and from the post-ribosomal supernatant as previously described[10,17].

Yoshida ascites hepatoma cells were harvested from the peritoneal cavity of Wistar rats, weighing 120–150 g, 3, 6 and 12 days after implantation of 1.1×10^8 cells/rat, obtained from the same donor animal. Cells were collected, purified and ruptured by homogenization as described[10].

The preparation of the ribosomal dissociation factor from ribosomes and from the post-ribosomal supernatant was as described for rat liver.

The assay of dissociation factor activity was performed at 25 °C with 0.3 mM Mg acetate and 100 mM KCl as described earlier[17]. The rate of dissociation was calculated as reported previously[6,10,17].

Results and discussion. The results are reported in the table. Same dissociation factor activity occurs in the ribosomal wash preparations of liver and ascites cells harvested 3 days after i.p. implantation. Then the activity of ascites hepatoma decreases. Cells at the stationary phase of growth[19], harvested 12 days after implantation, show considerably reduced dissociation factor activity in the high salt ribosomal wash preparations in respect to normal liver.

The post-ribosomal supernatant extracts from ascites hepatoma do not show dissociation factor activity on ribosome monomers, at least in present conditions, as compared to normal liver. These extracts promote the association of 40S and 60S particles into 80S ribosomes at low Mg^{2+} concentration, with no aminoacyl-tRNA and mRNA added. Association is more evident in cells at the stationary phase of growth and increases linearly with respect to added extract.

The observations here reported suggest the presence in ascites hepatoma cytosol of a factor(s) that promotes the association of subunit ribosomes into 80S monomers at low Mg^{2+} concentration. Association factors containing polyamines and a protein component have been described[20–23]. They are isolated together with dissociation factor and act at low Mg^{2+} concentration.

Association factors are able to reverse the splitting of ribosome monomers caused by dissociation factor. Recently, the protein component has been found to inhibit the Phe-tRNA binding to ribosomes and the synthesis of polyphenylalanine[24]. Preliminary work appears to indicate a similar effect also in present conditions.

1 The technical assistance of Miss M. Ravazzani and Miss L. Zingaretti is gratefully acknowledged.

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