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# *Echinococcus multilocularis*: In vitro interactions between protoscolices and Kupffer cells

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**Abstract** *Echinococcus multilocularis* protoscolices collected from experimentally infected jirds were incubated for 2 weeks in rat hepatic cell cultures cocultivated with or without feeder cells (BALB/c 3T3 and IAR 20). Scanning and transmission electron microscopy studies were performed during the course of the culture period. Kupffer cells (Kc) were seen adhering to the anterior and posterior ends of the protoscolices. Some protoscolices were fixed to the cell monolayer by a cluster of Kc adhering to the posterior end of the parasite. These cells were phagocytosing the glycocalyx and the electron-dense distal end of the microthrix of the protoscolex tegument. An alteration in the superficial tegumental cytoplasm with extensive mitochondrial damage was also noted. The properties expressed by Kc against protoscolices in vitro might also be relevant for proliferation of metacestodes in vivo.

## Introduction

Several mechanisms are involved in the spread of *Echinococcus multilocularis* within the intermediate host body: the differentiation of oncospheres into alveolar cysts after their migration from the mesenteric vein to the liver, the infiltration of host tissues by protrusions or buds of the germinative layer, the detachment of small parts of the buds comprising a few nuclei and their transport to the target organ (Eckert et al. 1983), and the cystic transformation of protoscolices extruded from primary cysts after cystolysis or rupture. In the case of secondary cyst development due to protrusion and spreading of protoscolices from the initial multivesicular cyst, this larval stage must be at least partly in contact with the endothelial barriers (Frank 1976).

Natural and experimental hosts may develop partial resistance to the installation and proliferation of the metacestode, and a variety of nonparenchymal hepatic cells may participate in the development of this resistance (Ali-Khan 1978). Using different experimental models, several workers have investigated the role of specific immunological reactions or nonspecific inflammation in the intermediate host (Rau and Tanner 1973; Araj et al. 1977; Baron and Tanner 1977) and the cytotoxic effect of protoscolices on surrounding tissues (Kirkpatrick and Svilenov 1987). Most of the work was performed with macrophages and lymphocytes (Rau and Tanner 1976; Baron and Tanner 1977) and, more recently, with endothelial cells (Kirkpatrick and Svilenov 1987). However, these cells can differ significantly from those that are likely to encounter the parasite in the liver, i.e., the Kupffer cells, and the receptors for parasites and the state of activation may be different (Crocker 1986).

Kupffer cells (Kc), which represent 90% of the reticuloendothelial system of the liver, are commonly regarded as the first line of defense against environmental and infectious agents (Wardlaw and Howard 1959) or parasites (Meis et al. 1983; Zachariou et al. 1986) transported by the blood to the liver. These cells have the ability to adhere to, to alter, and to phagocytose foreign particles (Seljelid 1980). Using hepatic cell cultures, we investigated the in vitro properties of Kc and the modifications that occurred at the Kc/protoscolex interface. These modifications were examined at the ultrastructural level.

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## Materials and methods

### Cell cultures

Two different types of mammalian cell culture were used in this study: hepatic cell cultures from BD VI rats cocultured with BALB/c 3T3 cells (embryonic mouse fibroblastic cells) or 10-day-old rat epithelial cells (IAR 20), which are necessary to maintain the differentiated state of hepatocytes. Hepatic cells were obtained from male BD VI rats (180–200 g weight) by a two-step perfusion method with collagenase (Seglen 1973) as previously described (Mesnil et al. 1987). Cells were collected in William's E medium and centrifuged for 4 min at 50 g. The cell sediment was washed twice in William's E medium supplemented with L-glutamine (2 mM/ml), penicillin (100 UI/ml), and streptomycin (100 µg/ml). Then, cells were collected in William's E medium supplemented as described above and with 10% fetal calf serum, counted, and estimated for viability using the trypan blue dye-exclusion test. For every cell-culture system, hepatic cells were resuspended in 3 ml of supplemented William's E medium and plated at a seeding density of $3 \times 10^6$ viable cells in 60-diameter petri dishes (Falcon). An approximately 10% content of non parenchymal cells, mostly Kc, was present in the cell suspension. The monolayer cultures were incubated at 37° C in a humidified atmosphere of 5% $CO_2$ 95% air.

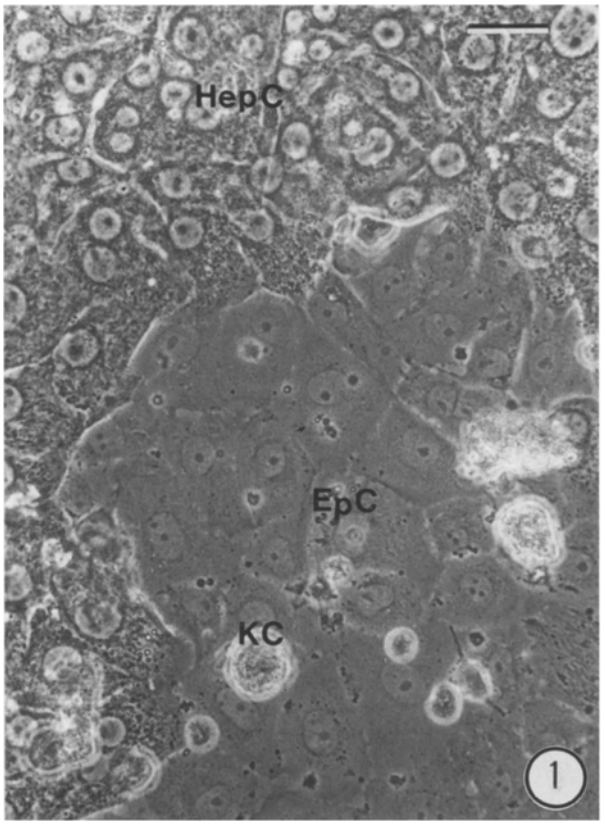
Embryonic mouse BALB/c 3T3 cells were obtained from Dr. T. Kakunaga (1973). At 3–4 h after the seeding of hepatic cells, $1.5 \times 10^6$ BALB/c 3T3 or IAR 20 cells were added to hepatic cultures. After a 24-h incubation period, necessary for cell confluence, culture medium was replaced by supplemented William's E medium with 10% heat-inactivated (56° C, 1 h) fetal calf serum, $3.5 \times 10^{-6}$ *M* hydrocortisone hemisuccinate, and 5 µg flucytosine/ml (Ancotil). L-Glutamine and antibiotics were added as described above. Thereafter, the culture medium was changed every 48 h. Cell viability was estimated using the trypan blue dye-exclusion test.

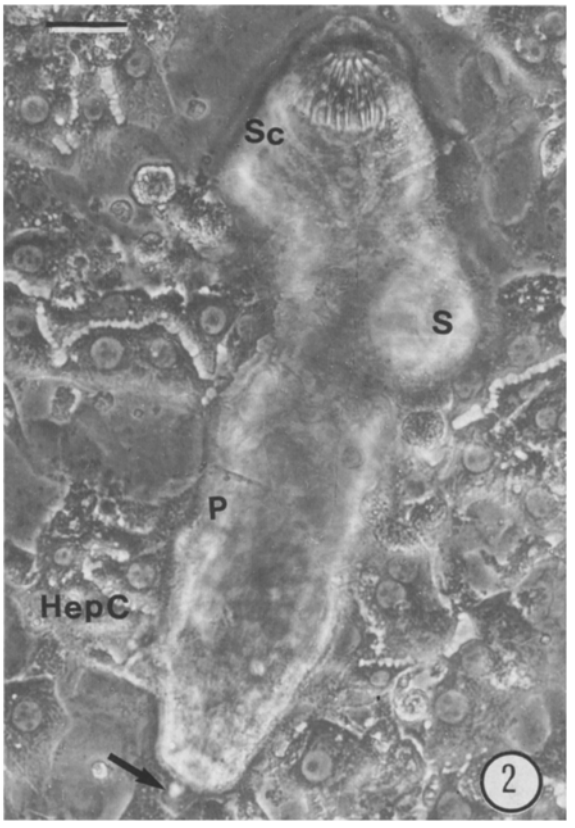
### Parasites

The protoscolices of *Echinococcus multilocularis* were recovered from jirds (*Meriones unguiculatus*) that had been infected intraperitoneally 3 months prior to the experiment. Protoscolices were separated from cyst components using gauze filtration. Contrary to the classic methods used for their isolation (Smyth 1979) and to avoid glycocalyx changes, protoscolices were obtained without enzymatic treatment of the cysts and without incubation in sodium taurocholate solution for evagination. Only unevaginated protoscolices, selected under lens, were used in the in vitro studies. From 100 to 120 protoscolices were placed in an Eppendorf centrifuge tube and washed for 5 min at room temperature, four times with 1 ml of Hanks' solution and then once with 1 ml of William's E medium. Each washing was followed by a 4-min centrifugation step at 70 *g*. The pellet was resuspended in 200 µ l of William's E medium. Approximately 100 protoscolices were added to each 48-h cell culture. Cultures were maintained in a humidified atmosphere of 5% $CO_2$ 95% air at 37° C for 14 days.

Host-cell interactions were examined daily for a fortnight and the progress of the reaction was monitored using an inverted Olympus CK2 photomicroscope. Specimens were also processed for scanning electron microscopy and transmission electron microscopy. The eosin exclusion test according to Smyth and Barrett (1980) was used to assess the viability of the protoscolices.

**Fig. 1** Localization of Kupffer cells (*KC*) by endogenous peroxidase in a culture of adult rat hepatic cells (*Hep C*) cocultured with IAR 20 rat epithelial cells. (*Ep C.*) On the micrograph, the spherical Kupffer cells (*KC*) are characterized by a central yellow coloration. Bar = 30 µm. **Fig. 2** Adhesion of a protoscolex to a monolayer of adult rat hepatic cells cocultured with embryonic mouse cells. The evaginated protoscolex (*P*) is attached to the cell monolayer (*Hep C*) by its posterior end (*arrow*). (*Sc* Scolex, *S* sucker) Bar = 30 µm



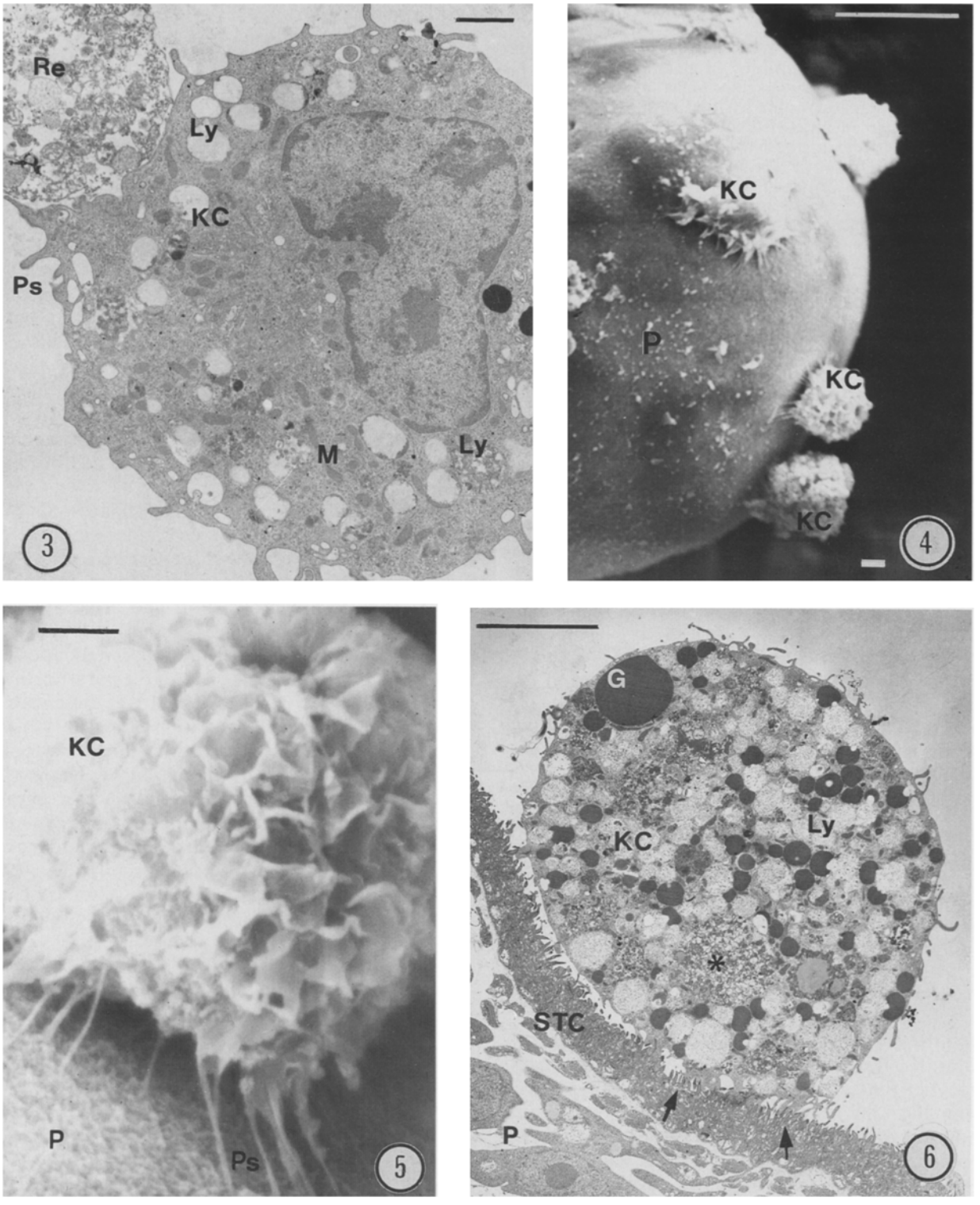



**Fig. 3** Electron micrograph of a control spherical Kupffer cell (*KC*) showing phagocytic activity: cellular remnants are maintained in close contact with *KC* membrane by peripheric broad pseudopodia (PS). The cell cytoplasm exhibits numerous small mitochondria (*M*): electron-lucent lysosomes (*Ly*), some of which contain dense amorphous material and a low nuclear/cytoplasmic ratio. Bar = 1 μm. **Fig. 4** Spherical or spread Kupffer cells (*KC*) adhering to the surface of an *Echinococcus multilocularis* protoscolex (*P*). Bar = 5 μm. **Fig. 5** Aspects of a highly activated Kupffer cell (*KC*) adhering to a protoscolex (*P*), with many ruffles appearing on the whole cell surface and numerous cytoplasmic processes (*Ps*) extending toward the parasite. Bar = 1 μm. **Fig. 6** Electron micrograph of a strongly stimulated Kupffer cell (*KC*) in contact with the parasite (*P*); numerous pseudopodia are interdigitating with spinous microtriches (*arrows*). The cell cytoplasm is filled with lysosomes (*Ly*), multivesicular bodies (*), and dense granules (*G*). (*STC* Parasite tegument) Bar = 5 μm

### Kc identification

For Kc identification according to Fahimi (1970), with monolayer cultures were fixed with a mixture of 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.3) for 15 min. After three brief immersions for 5 min each in phosphate buffer and several changes in TRIS-HCl buffer for 30 min, cultures were incubated for 20 min in medium containing 10 mg of 3,3'-diaminobenzidine tetrahydrochloride (DAB) in 10 ml of 0.5 *M* TRIS-HCl buffer (pH 7.6) and 0.02% $H_2O_2$. After being washed with TRIS-HCl buffer, the cultures were observed using an inverted microscope. Kupffer cells are characterized by a central yellow coloration.

### Scanning electron microscopy

Cell cultures with protoscolices were rinsed with 3 ml of prewarmed (37° C) William's E medium, fixed for 60 min in a 2% glutaraldehyde-0.5% paraformaldehyde solution in 0.08 *M* (PBS, pH 7.3), and rinsed twice with the same buffer. The material was postfixed for 60 min in 1% osmium tetroxide in 0.08 *M* PBS (pH 7.3), rinsed in distilled water, and dehydrated in an increasing ethanol series. Following dehydration, the alcohol was replaced by graded solutions of freon and absolute alcohol. Petri dishes were dried at room temperature and cut off. Specimens were coated with gold/palladium wire. A JEOL JSM 35 CF microscope was used for scanning examination.

### Transmission electron microscopy

Cell cultures with protoscolices and control cell cultures without protoscolices, previously rinsed, were fixed in a 2% glutaraldehyde-0.5% paraformaldehyde solution in 0.1 *M* PBS (pH 7.3) for 30 min (control cultures) or and 75 min (cell cultures containing protoscolices). Petri dishes were then rinsed twice for 15 min in 0.2 *M* PBS (pH 7.3) and postfixed for 75 min at room temperature in 1% osmium tetroxide in 0.1 M PBS (pH 7.3). Then, they were quickly washed in distilled water and dehydrated in an ethanol series graded up to 100%. Specimens were embedded in an Epon 812 mixture without treatment with organic solvent. Ultrathin sections (800–1,000 A) were stained in uranyl acetate and lead citrate and examined under a JEOL XC III microscope operating at 80 kV.

## Results

### Microscopical findings

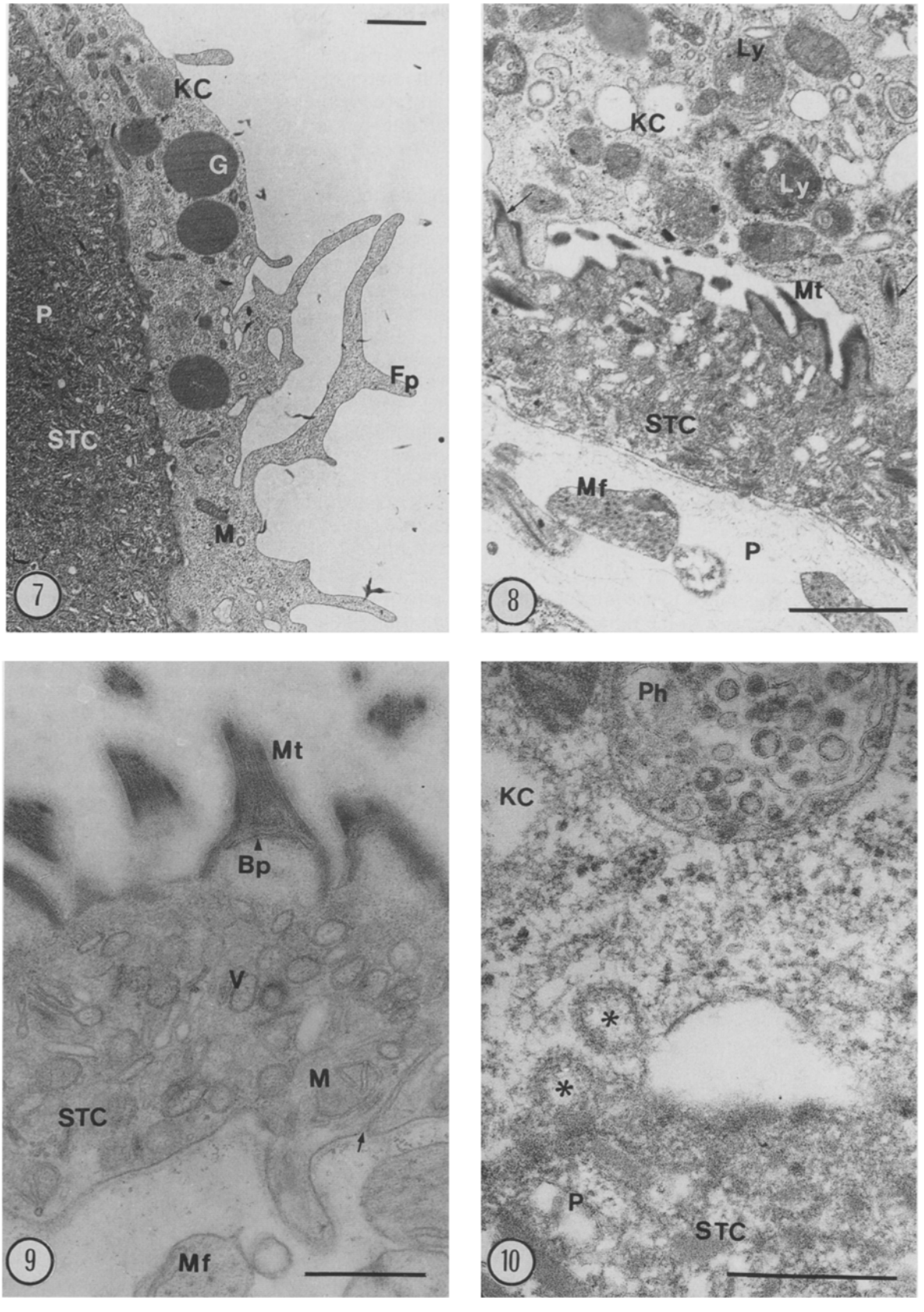
Among the cells observed free in the control-cell culture medium, Kc were characterized by their morphology, phagocytic function, and positive DAB-peroxidase staining (Fig. 1). Within 24 h of the addition of parasites, some Kc were seen attached to the protoscolices. Adhesion of free Kc to the parasite increased with time and was observed on unevaginated and evaginated protoscolices. After 48 h, some protoscolices were fixed by their posterior ends to Kc still adhering to the monolayer (Fig. 2). The protoscolices remained attached throughout the culture period. During these two phenomena-adherence of free cells to the parasite or adhesion of the parasite to the monolayer- and parasites were fully viable and their motility was maintained.

### Ultrastructural observations of Kupffer cells

Control Kc showing phagocytic activity had a spherical shape, with broad pseudopodia being directed toward cellular debris derived from nonsurviving cells and numerous short filipodia radiating around the cell (Fig. 3). The nuclear/cytoplasmic ratio was low. The nucleus was eccentrically positioned, with an irregular outline showing some indentations; mottled chromatin was located near the nuclear membrane and the nucleolus was clearly visible. The cytoplasm of Kc contained small, round or elongated mitochondria with complete transversal cristae, rough endoplasmic reticulum that was not clearly visible, free ribosomes scattered throughout the cytoplasm, and numerous Golgi vesicles of different size located near the cytoplasmic membrane. Many electron-lucent lysosomes as well as some dense granules were also observed at the periphery of the cell cytoplasm. Confluence of phagolysosomes was occasionally observed, forming larger phagosomes located opposite the nucleus.

In cell cultures containing protoscolices, Kc of two different morphologies were seen adhering to the parasite (Fig. 4). These were classified as spherical or spreading Kc. The spherical Kc, adhering to the parasite (Fig. 5), showed numerous short and broad pseudopodia inserted into the microtriches of the protoscolex (Fig. 6). Their nuclei were always observed opposite the site of attachment to the surface of the parasite. In comparison with the control spherical Kc, these cells presented a remarkable accumulation of lucent lysosomes, dense granules, and lipid inclusions in their cytoplasm. A high degree of this Kc stimulation by the parasite is represented in transmission electron microscopical observations (Fig. 6) where the cytoplasm of Kc is entirely filled. Spreading Kc were molded onto the surface of the protoscolices (Fig. 7). Long, branched filipodia could be seen on the cell surface opposite the site of attachment to the parasite. The morphology of the pseudopodia

**Fig. 7** A spreading Kupffer cell (*KC*) molded onto the posterior end of a protoscolex (*P*). Note the absence of pseudopodia face to the parasite surface. The cell cytoplasm contains numerous elongated mitochondria (*M*) and dense granules (*G*). Long, branched filipodia (*Fp*) are present opposite the attachment site. (*STC* Parasite tegument) Bar = 1 μm. **Fig. 8** High magnification of a Kupffer cell (*KC*) spreading on the tegument (*STC*) of the anterior portion of a protoscolex (*P*). Note the phagocytosis and internalization (*arrows*) of spinous microtriches (*Mt*). (*Mf* Muscular fiber, *Ly* lysosome) Bar = 1 μm. **Fig. 9** Normal tegument at the anterior portion of the parasite. The cytoplasmic membrane is lined by spinous microtriches (*Mt*); the cytoplasm contains a substantial population of membrane-bound vesicles (*V*). Mitochondria (*M*) are enclosed in spaces delimited by basement-membrane indentations (*arrow*). (*Mf* Muscular fiber, *Bp* basal plate, *STC* parasite tegument) Bar = 0.25 μm. **Fig. 10** High magnification of a Kupffer cell (*KC*) adhering to the tegument of the posterior portion of a protoscolex (*P*). Note the absence of microtriches at the surface of the tegument; the Kupffer cell is attached to the tegument (*STC*) in a few places; micropinocytic vesicles (*) are visible face to the parasite tegument. (*Ph* Phagosome) Bar = 0.25 μm




Figs. 7–10

depended on the Kc localization on the parasite. When they adhered to the anterior extremity of the protoscolex (Fig. 8), the cells formed cytoplasmic processes interdigitating with the microtriches of the tegument. When they were fixed to the posterior end of the parasite, covered with knob-like structures, the pseudopodia were absent and numerous micropinocytic vesicles were observed (Fig. 10). The nucleus, located in the middle of the cell, was elongated. As compared with the control Kc, its structure was practically unchanged. The cell cytoplasm contained elongated mitochondria with cristae lying parallel to the cells major axis and many lysosomes, most of which contained dense amorphous material. Some multivesicular bodies, which were not observed in controls, were also found in the cytoplasm of this spreading cell (Fig. 10).

### Ultrastructural observations of the parasite

As compared with normal protoscolex structures (Fig. 9), where the cell coat appeared as a fine, thread/like network containing few vesicles that was particularly dense in the posterior region of the protoscolex glycocolyx disappeared during the culture period but this normal process was accelerated by Kc phagocytosis. Parasite alterations were limited to the integument, e.g., glycocalyx disappearance face to the Kc and partial destruction of the microthrix. Microthrix tips were separated from their basal parts at the pentalaminar baseplate level (Fig. 8). The depth of the parasite syncytial tegumental cytoplasm was not modified, but the mitochondria observed lining the basement membrane and in subtegumental cells (Fig. 9) were extensively damaged, showing disintegration of the matrix and cristae. The indentations of plasma membrane with the basement membrane were less numerous. No alteration in the muscular fibers was noted, which is consistent with the persistent mobility of the parasite during the experiment.

### Ultrastructural observations of the parasite-Kc interface

At the surface areas where contact between spherical Kc and the tegument of the anterior portion of the parasite occurred, the microtriches isolated from the parasite by cytoplasmic processes of the Kc were internalized in endocytic vesicles (Fig. 8) and were later observed in larger phagosomes. Pseudopodia of the Kc were retracted. The integument of the parasite looked rubbed. With regard to parasite-cell contact between the anterior end of the parasite and the spreading Kc, only the leading lamella formed pseudopodia at the cell periphery that interdigitated with the microthrix tips (Fig. 8). At the posterior end of the parasite, which lacks microtriches, the Kc membrane was in close contact with the parasite tegument except where the glycocalyx was persisting. Numerous micropinocytic vesicles were seen at the cell surface (Fig. 10).

## Discussion

The hepatic cell-protoscolex coculture model shows that among parenchymal and nonparenchymal liver cells, Kc are the most relevant because of their particular interaction with the parasite. Our results provide evidence for rapid (within 24 h) adherence and activation of Kc to protoscolices; this delay of adherence is shorter than that described for peritoneal macrophages (3–4 days, Rau and Tanner 1973; Baron and Tanner 1977) but longer than that reported for human endothelial cells (30 min, Kirkpatrick and Svilenov 1987). As noted by Baron and Tanner (1977) with peritoneal macrophages, Kc were not distributed uniformly over the surface of the protoscolices; they tended to cluster, especially around the posterior pole of the protoscolex. This preferential localization is consistent not only with the more dense glycocalyx structure on this posterior pole (McManus and Barrett 1985) but also with its longer persistence during in vitro culture (Morseth 1967).

Rau and Tanner (1973) and Baron and Tanner (1977) have observed a more extensive adherence to the surface of protoscolices with peritoneal cells collected from infected animals than with cells obtained from uninfected control animals, suggesting a correlation between the intensity of the cell-adherence phenomenon and an immunological factor. The same phenomenon was reported by Zachariou et al. (1986) using an in vitro Kc-*Schistosoma mansoni* model. Kirkpatrick and Svilenov (1987) have reported that serum is required to induce protoscolex adherence to cultured endothelial cells. In our experiments, inactivation of normal fetal calf serum at 56° C for 1 h did not prevent the adherence of Kc. These findings imply that Kc can interact with protoscolices in the same way as they would with other foreign surfaces or that Kc cell adherence may be effected via immunoglobulins that have bound to the tegumental membrane of the parasite prior to incubation in vitro as suggested by Rau and Tanner (1976) for peritoneal cells. This hypothesis is consistent with and supports the observation that host immunoglobulins are a normal constituent of the cyst fluid that bathes the protoscolices (Varela-Diaz and Coltorti 1972).

The process of phagocytosis by Kc occurred very quickly after their adherence to the protoscolices; the former process involved a faster elimination of the glycocalyx of the parasite, whose remains were seen included in phagosomes. As previously observed by Engelkirt et al. (1981) in an in vitro rat- *Taenia taeniaeformis* model, the apical portion of the microthix (spinous or knob-like) is separated from its basal part during the phagocytosis process and is internalized by the Kc. In addition to adherence and phagocytosis, the Kc damaged the superficial syncytial cytoplasm and caused mitochondrial alteration. The mobility of the parasites was maintained during the course of the experiment.

Indeed, although our morphological data provide evidence that Kc induce alterations in the integument,

they do not indicate that the action of Kc is sufficient to kill the parasite. As has been suggested for *Schistosoma mansoni* (Capron et al. 1978), cooperation between different types of cells may be necessary to obtain this effect. That our cell-protoscolex coculture system was a nondynamic model that did not involve proliferation or cellular recruitment could also explain the limited tegumental damage observed on the parasite. In any case, one of the most interesting aspects of this study was the demonstration of a cell-adherence reaction of Kc from the rat liver to *Echinococcus multilocularis* protoscolices in vitro that was destructive to the distal tegument.

Our experiments demonstrate a role in vitro for Kc, but the role in vivo of this cell type remains to be established. It is likely that not only protoscolices but also other stages of the parasite such as oncospheres have the ability to activate reticuloendothelial cells and that Kc might therefore also play a role in the defense against early infections with *E. multilocularis*.

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## References

Ali-Khan Z (1978) *Echinococcus multilocularis*: cell mediated immune response in early and chronic alveolar murine hydatidosis. Exp Parasitol 4:157–165

Araj GF, Matossian RM, Malakian AH (1977) The host response in secondary hydatidosis of mice. II Cell mediated immunity. Z Parasitenkd 52:31–38

Baron RW, Tanner CE (1977) *Echinococcus multilocularis* in the mouse: the in vitro protoscolicidal activity of peritoneal macrophages. Int J Parasitol 7:489–495

Capron M, Capron A, Torpier G, Bazin H, Bout D, Joseph M (1978) Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of Ig G2a antibody and role of mast cells. Eur J Immunol 8:127–133

Crocker RR (1986) Macrophage parasite interactions in vitro: potential use of extracellular matrix. Parasitol Today 2:148–150

Eckert J, Thompson RCA, Mehlhorn H (1983) Proliferation and metastase formation of larval *Echinococcus multilocularis*. I Animal model, macroscopical and histological findings. Z Parasitenkd 69:737–748

Engelkirk PG, Williams JF, Signs MM (1981) Interaction between *Taenia taeniaeformis* and host cells in vitro. Rapid adherence of peritoneal cells to strobilocerci. Int J Parasitol 11:463–474

Fahimi HD (1970) The fine structural localization of endogenous and exogenous peroxidase activity in Kupffer cells of rat liver. J Cell Biol 47:247–262

Frank W (1976) Parasitologie. Eugen Ulmer, Stuttgart

Kakunaga T (1973) A quantitative system for assay of malignant transformation by chemical carcinogens using a clone derived from BALB/c 3T3. Int J Cancer 12:463–473

Kirkpatrick CJ, Svilenov D (1987) Studies on the adhesion of protoscolices from *Echinococcus multilocularis* and *E. granulosus* to artificial substrates and human endothelial cells in vitro. Parasitol Res 74:61–68

McManus DP, Barrett NJ (1985) Isolation, fractionation and partial characterization of the tegumental surface from protoscolices of the hydatid organism *Echinococcus granulosus*. Parasitology 90:111–129

Meis JFG, Verhave JP, Jap PHK, Meuwissen JHET (1983) An ultrastructural study on the role of Kupffer cells in the process of infection by *Plasmodium berghei* sporozoites in rats. Parasitology 86:231–242

Mesnil M, Fraslin JM, Piccoli C, Yamasaki H, Guguen-Guillouzo C (1987) Cell contact but not junctional communication (dye coupling) with biliary epithelial cells is required for hepatocytes to maintain differentiated functions. Exp Cell Res 173:524–533

Morseth DJ (1967) Fine structure of the hydatid cyst and protoscolices of *Echinococcus granulosus*. J Parasitol 53:312–325

Rau ME, Tanner CE (1973) *Echinococcus multilocularis* in the cotton rat. The effect of pre-existing subcutaneous cysts on the development of a subsequent intraperitoneal inoculum of protoscolices. Can J Zool 51:55–59

Rau ME, Tanner CE (1976) *Echinococcus multilocularis* in the cotton rat: the in vitro protoscolicidal activity of peritoneal cells. Int J Parasitol 6:195–197

Seglen PO (1973) Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. Exp Cell Res 82:391–398

Seljelid R (1980) Properties of Kupffer cells. In: Van Furth R (ed) Mononuclear phagocytes: functional aspects. Martinus Nijhoff, The Hague

Smyth JD (1979) *Echinococcus granulosus* and *E. multilocularis*: in vitro culture of the strobilar stages from protoscolices. Angew Parasitol 20:137–147

Smyth JD, Barrett NJ (1980) Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans R Soc Trop Med Hyg 74:649–652

Varela-Diaz VM, Coltorti EA (1972) Further evidence of the passage of host immunoglobulins into hydatid cysts. J Parasitol 58:1015–1016

Wardlaw AC, Howard JG (1959) A comparative survey of the phagocytosis of different species of bacteria by Kupffer cells. Br J Exp Pathol 40:113–117

Zachariou Z, Kubelka CF, Rauterberg EW, Krug M, Knierim M, Ruppela A (1986) New properties of Kupffer and endothelial cells isolated from rats infected with the parasite *Schistosoma mansoni*. In: Kirn A, Knook DL, Wisse E (eds) Cells of the hepatic sinusoid, vol 1. Kupffer Cell Foundation, RIJSWIJK, The Netherlands, pp 329–334