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HISTOPATHOLOGICAL DIAGNOSTICS OF LIVER AMOEBIASIS IN CAMBODIA

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Abstract. Results of histopathological examination of a solitary chronical amoebic abscess in the liver of a patient from Cambodia are described. The abscess was at a proliferously reparative phase and *Entamoeba histolytica* was detected at the border between the necrosis and inner wall of abscess, as well as in the proper non-specific granulation tissue of the inner layers of abscess capsule. A differential diagnosis of the amoebic abscess from similar parasitary or pseudoparasitary liver lesions and differential diagnosis of amoebae in histological sections are given. For orientation examinations of the liver tissue for the presence of amoebae the authors recommend the impregnation after Grocott and staining with Goldner's trichrome for a more detailed evaluation of histological sections.

Entamoeba histolytica is a frequent parasite in Cambodia. Its distribution in the population amounts to 17—30 % in relation to the age of the examined patients, with maximum incidence in the age-group of 10—14 years. Microscopical examinations of 235 cases of intestinal amoebiasis revealed erythrophagous trophozoites, which is an evidence of the infectivity of the strain (Giboda 1985). Indochine has always been considered the main focus of amoebic liver abscess so that the French authors reported of hepato-trophic amoebae of the Far East (Eldson-Dew 1968).

The participance of *E. histolytica* in the aetiology of liver abscesses was demonstrated also in the hospital of Takeo (People's Republic of Cambodia). The histopathological diagnostics of the amoebae is dealt with in our Department.

MATERIAL AND METHODS

Clinical observations. Blood was taken from two patients with liver abscesses. The serum fixed in Merthiolate was examined in Czechoslovakia by the methods of indirect haemagglutination (IHA) and countercurrent immunoelectrophoresis (CIEP) using commercial antigens (Behringwerke, FRG). A negative result of serodiagnostical examination was obtained in the patient with the abscess localized in the left liver lobe. In the other patient, with the abscess localized in right liver lobe, cysts of *E. histolytica* were found in the stool (mixed infection with *Entamoeba coli* and *Endolimax nana*) and the results of serological examinations were as follows: IHA 1 : 2048, CIEP positive with undiluted serum. Microscopical examinations for the presence of amoebae in the abscess contents were negative in both patients.

A third patient was admitted to the hospital for marked gradual weight losing. A severe cachexia, anaemia and dehydration were found in him. The liver lobe overlapped by 4 fingers the right costal margin. A slightly fluctuating resistance of the size of man fist was palpable through the abdominal wall beyond the right costal margin. The temperature was 37 °C and the stool was regular and formed. *Pentatrichomonas hominis* and *Ancylostoma duodenale* were demonstrated by parasitological examination of the stool. Since the patient's health condition continuously deteriorated, he was operated after treatment with Flagyl.

When the abdominal cavity was opened, a yellow-green dense purulent fluid with a brown tint at some sites flew out from the space above the convexity of the right liver lobe. A perforated abscess measuring 8 × 5 × 4 cm was found at the right side of the right liver lobe. Fibrin pseudomembranes and foci of pus were found in the right subfrenum. The Douglas's space contained a small amount of inflammatory seat. Palpation of other organs of abdominal cavity did not reveal any changes (operator Dr. Ladislav Frühauf). The abscess contents were collected during the operation and examined microscopically in a native preparation. Erythrocytes, single cells and

remnants of necrotic tissue were found, but no amoebae or bacteriae were present. Another material for examination was scraped off from the abscess wall covered with a dense inflammatory layer and put into physiological saline. A large number of amoebae accumulated in clusters were detected in the native preparation. They moved rapidly in one direction and contained phagocytized erythrocytes.

Histological method. Portions of the abscess were fixed in 10 % neutral formol and processed by a standard paraffin technique. Partial series of histological sections were made from the excisions. Haematoxyline-eosine, Van Gieson's method, Van Gieson-elastica method, Goldner's green trichrome, PAS and Grocott's modification of Gömöri's impregnation method were used for staining. The differential diagnosis of the origin of the abscess wall was made using some methods of Štěrba and Šlais (1972, 1974) and Štěrba (1978).

RESULTS

When the liver abscesses were emptied and the material for the diagnosis was removed, it was evident that the wall of the abscess was always uneven, broken and stiffened with connective tissue encapsulation at the outside. The contents were granular, semiliquid and of yellow-green or slightly brown colour.

Histological examination revealed a progressed chronical abscess consisting of necrotic tissue detritus and leukocytes. The abscess cavity contained granular inflammatory exudate with necrotic leukocytes and with the admixture of numerous erythrocytes and disintegrated single eosinophiles. The inner surface of the abscess wall (Plate I, Figs. 1 and 3) contained remnants of fibrin and inflammatory elements, particularly histiocytes, neutrophilic leukocytes, single eosinophiles and fibroblasts, which made up a non-specific granulation tissue forming an uneven layer. Towards the periphery, this layer gradually turned to a mature collagenic connective tissue (Plate I, Figs. 1—4; Plate II, Fig. 1). The amoebae were found in the zone between the pyogenic membrane, but also in the proper non-specific granulation tissue and, particularly, in adjacent necrotic matters (Plate I, Figs. 2—4; Plate II, Figs. 2—4). The amobae, which were well visible especially after staining by Goldner's and Grocott's methods, could be identified as *Entamoeba histolytica* on the basis of their sizes (20 to 30 μm) and other characters. In histological sections, they were more or less oval, exhibited a marked erythrocytophagia, and contained numerous vacuoles or remnants of erythrocytes including the pigment from their disintegration.

The non-specific granulation tissue matured towards the periphery to layers of collagenic connective tissue which was deposited in form of lamellae at the periphery of the abscess. Due to the gradual resorption, the connective tissue not only matured, but also gradually folded (Plate II, Fig. 1). In some parts the amoebae adhered directly to the maturing collagenic connective tissue of the abscess envelope (Plate II, Fig. 3).

A peculiar feature of the abscess was the presence of traces of hepatocytes. In the focus of colliquated necrosis, there were, in addition to disintegrated or strongly regressively changed cells, even connective tissue remnants of vessels and portal fields. Cavities made by cholesterol crystals were sometimes found at some places.

DISCUSSION

In the case studied by us a chronic amoebic abscess of proliferatively reparative character was involved. The resorption of the contents is indicated by gradual scarring leading to wrinkling of the maturing connective tissue (folding and resulting retraction) (Plate II, Fig. 1) or to formation of histiocytes with a foamy light plasma, gradual resorption of disintegrated cells and formation of foci of pseudoxanthomas.

The larger number of free erythrocytes or erythrocytes resorbed by amoebae results from their release from the capillaries of the non-specific granulation tissue and from

damaged portal veins. At the acute phase, the presence of eosinophilic leukocytes is usually a part of inflammatory changes and reaction to the parasite and their occurrence at a later period results from a common phenomenon, i.e. the presence of eosinophilic leukocytes and eosinophilic histiocytes is a manifestation of the reparative phase of the proliferative component of healing and encapsulation of the abscess. Together with the presence of foci of lymphoblasts and lymphocytes it is typical and according to many authors it is a manifestation of the immune reaction (Schwartz 1966, El-Hashimi 1971, Schwartz et al. 1974, Štěrba 1978).

The diagnostics of parasites in histological sections is very important. The determination of protozoans is sometimes difficult even if the structure of parasites is preserved, as it is evident from the course of epidemic spreading of meningoencephalitis in North Bohemia in 1962—1965 (Červa et al. 1968) or the set of protozoal diseases reported by Vortel and Štěrba (1983).

Of the parasitic lesions, the regressively changed cysts of echinococci most closely resemble the liver amoebic abscesses and can be mistaken for them at differential diagnostics (Štěrba and Šlais 1974, Štěrba 1978, Štěrba and Prokopič 1981, Prokopič et al. 1983). However, they can be reliably, though sometimes very hardly, differentiated on the basis of the characters described in the above-cited papers (Štěrba and Miláček 1983).

Of the non-parasitic lesions it is necessary to distinguish particularly regressively changed serous and other liver cysts (Štěrba and Šlais 1972, 1974, Štěrba 1978) and to exclude the presence of amoebae. An important factor in these liver structures is the enzymatic environment in the infected focus, which is influenced by the organ cells destructed by the parasite, tissue fluid, exudation of the fluid, and exudative and infiltrating cells. Particularly the eosinophilic leukocytes and their number affect significantly the character of the focus upon which depends the further fate of the infected tissue (Šlais 1974, Štěrba 1987).

Sepulveda (1982), who studied amoebiasis caused by *E. histolytica*, observed two important features of the intestinal and liver lesions. In agreement with some other authors (Prathap et al. 1970, Aquirra-Garcia 1970, Lushbangh et al. 1980 ex Sepulveda 1982) he found that in the acute phase, tissue necrosis prevails over inflammatory changes which are visible in later phase. According to Peréz-Tamayo and Brandt (1971), Landa et al. (1976) and Sepulveda (1982) another characteristic feature is the healing of even extensive amoebic lesions, as abscesses, without scarring ad integrum. In our case, there was a conspicuous gradual resorption of the abscess and scarring, the terminal phase of which, according to our experience and that of other authors, is either the scar or liver node the origin of which must be always demonstrated (Torres 1967, Peréz Tamayo and Brandt 1971, Štěrba and Šlais 1972, 1974, Mohr 1973, Shing-Shen Lin and Schwartz 1976, Štěrba 1978, Jimenez 1981). From the prognostic viewpoint, no marked damage of the liver function occurs during healing of these lesions, as well as of amoebic abscesses in the liver.

The amoebic infection can be differentiated from other similar structures in the histological material on the basis of the following characters: a) The shape of the trophozoites is not constant for two reasons. The trophozoite may be cut when the histological sections are prepared and it becomes slightly rounded after fixation. Its shape is not elongated in one direction, as it is visible in the native scrapings from the abscess wall (or from the mucosa of large intestine). b) Not all of the amoebic trophozoites contain phagocytized erythrocytes and therefore not even erythrophagocytosis can be considered constant. c) The single constant character is the structure of nucleus of *E. histolytica*. It is spherical, measuring 3—4 μm in diameter, and chromatin granules are distributed at the periphery of the nuclear membrane on its inner side. The karyosome, or endosome, is small,

dotted and situated centrally. Lamy and Crignon (1972) regard the position of the karyosome as a significant specific character. Of the 1 000 examined trophozoites from different geographical regions, in 85 % the karyosome was situated centrally and in 15 % excentrically.

A comparison of the results of staining by different histological methods suggests that the impregnation after Grocott is very suitable for orientation examinations. The amoebae are visible at the first sight and can be easily distinguished from similar structures in the surrounding tissue. For a more detailed examination including the studies of the morphology of nucleus and cytoplasmic structures, the staining with green trichrome after Goldner can be recommended.

ГИСТОПАТОЛОГИЧЕСКАЯ ДИАГНОСТИКА АМЕБИАЗА ПЕЧЕНИ В КАМПУЧИИ

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Резюме. Описано гистопатологическое обследование одиночного хронического амебного абсцесса печени у больного из Кампучии. Абсцесс находился в пролиферативно-репаративной фазе и амеба *Entamoeba histolytica* была обнаружена между некрозом и внутренней стенкой абсцесса, а также в неспецифической грануляционной ткани внутренних слоев оболочки абсцесса. Приводится дифференциальный диагноз амебного абсцесса от похожих паразитических и псевдопаразитических поражений печени и дифференциальный диагноз амеб в гистологических срезах. Для ориентационного обследования ткани печени на присутствие амеб автори рекомендуют импрегнацию по Грокоту и для более подробной оценки гистологических срезов и амеб окраску трихромом Гольднера.

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RECORD OF DERMOCYSTIDIUM BRANCHIALE LEGER, 1914 IN SALMO TRUTTA M. FARIO IN SOUTH BOHEMIA

Dermocystidium branchiale Leger, 1914, which was only supposed to occur in our country (Ergens R., Lom J., Causative agents of parasitic diseases of fish, Academia, Praha, 284 pp., 1970, in Czech), was found on gills of *Salmo trutta* m. *fario* during studies on parasitic protozoans in fish in South Bohemia.

The systematic positon of the genus *Dermocystidium* is not quite clear. It was previously placed to Haplosporea, but at the present time, it is regarded as a fungal organism with an unclear development. The infection with *Dermocystidium* in fish is manifested by the formation of cysts on gills or skin. Of the species forming cysts on fish gills, *D. vejdovskyi* Jírovec, 1939 (Arch. Protistenk. 92: 137—146, 1939) was recorded in pike and *D. cyprini* Červinka et Lom, 1974 (J. Fish Biol. 6: 689—699, 1974) in carp. *D. percae* Reichenbach—Klinke, 1949 (Ergens R., Lom J., 1970), which belongs to the skin species, was found on perch.

Some of *Dermocystidium* species are known to produce a pathogenic effect on their hosts. For example, Hoshina and Sahara (Bull. Japan. Soc. Sci. Fish 15: 825—829, 1950) observed a marked reaction of the host organism (*Cyprinus carpio*) and decreased vitality after infection with the skin species *D. koi*. Červinka et al. (J. Fish Biol. 6: 689—699, 1974) reported a mass death of carp fry in some pond systems in South Bohemia, which was caused by *D. cyprini* infection on gills. The unfavourable effect of *Dermocystidium* species on young eel in breeding farms was described by Greuel et al. (Tierärztl. Prax. 7: 97—100, 1979) and the causative agent was identified as *D. branchiale*, originally described from *Salmo trutta* m. *fario*. Other authors suppose that a species specificity exists in *Dermocystidium*. For example, Elkan (Nature 196 (4858): 958—960, 1962) or Reichenbach—Klinke (Verh. Dtsch. Zool. Mainz, Leipzig, 126—132, 1949) state that in the locality where several fish species live together, only one of them is usually infected by a *Dermocystidium* species.

Dermocystidium forming cysts on the gills of *S. trutta* m. *fario* was described from Northern Ireland by Leger (C. r. Acad. Sci. Paris 158: 807, 1914), who named it *Dermocystidium branchialis*. In the territory of our country, this species was found for the first time on gills of trout from Borovnice brook in South Bohemia in spring 1980. The cysts are white, spherical, measuring 0.3—0.5 mm in diameter. The wall of young cysts is homogeneous and about 0.5 μm thick, whereas in older, mature cysts, it is not very solid and easily ruptures. The inner space of the cyst is not divided by

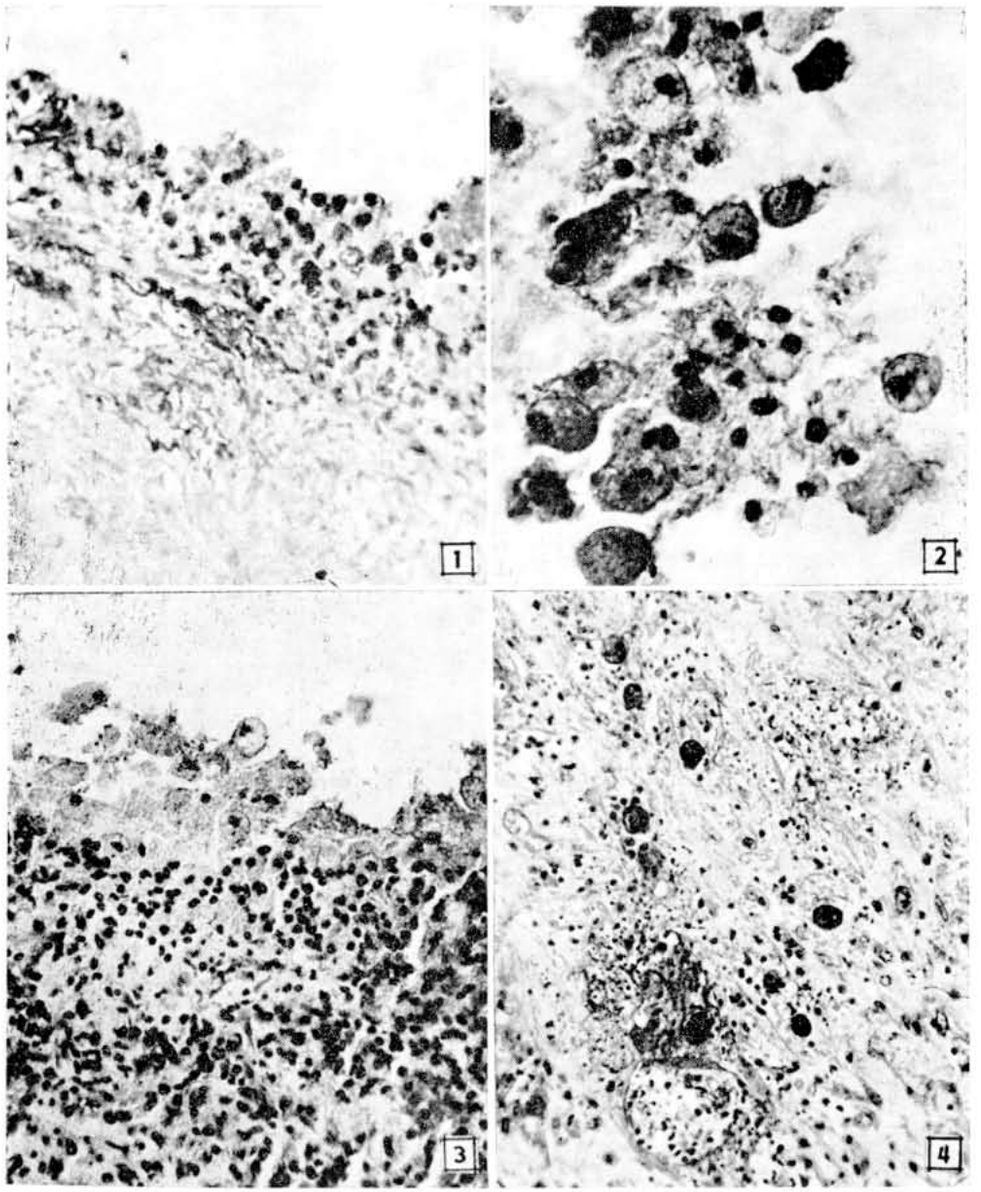

Fig. 1. Non-specific granulation tissue with amoebae and gradual maturing of connective tissue at the periphery of abscess. Grocott (×120). **Fig. 2.** Detail of inflammatory exudate with amoebae and single eosinophilic leukocytes. Goldner's green trichrome (×500). **Fig. 3.** Inflammatory rim with amoebae on the inner side of abscess. Haematoxyline-eosine (×200). **Fig. 4.** Granulation tissue with amoebae. Grocott (×165).

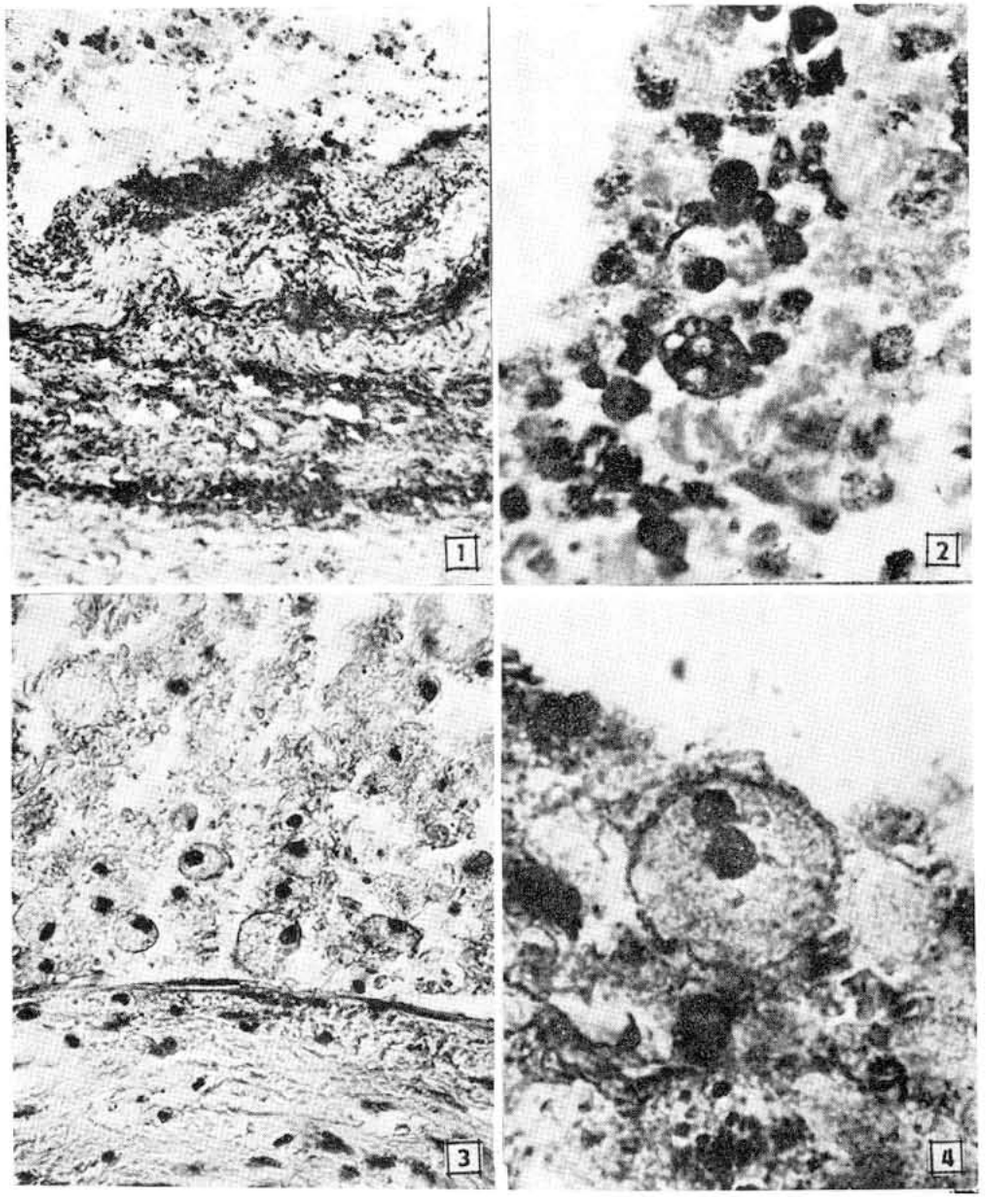

Fig. 1. Gradual maturing and folding of the connective tissue in the wall of amoebic abscess. Haematoxyline-eosine (×35). **Fig. 2.** Amoebae in the abscess contents. Grocott (×500). **Fig. 3.** Maturing connective tissue in the abscess wall and necrotic contents of the abscess with amoebae. Haematoxyline-eosine (×360). **Fig. 4.** Detail of amoeba with marked erythrocytophagia. Goldner's green trichrome (×1,000).