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Brief Communication

ON THE STRUCTURE OF INTESTINAL EPITHELIUM OF FASCIOLA HEPATICA DURING IN VITRO CULTIVATION

The normal ultrastructure of the intestinal epithelium of F. hepatica has been described in detail e. g. by *Thorsell & Björkman* (1965). However, experimentally produced pathological phenomena of the epithelium have not been investigated, though such studies might yield further information on the physiological function of the epithelial cell. Light and electron microscopy of the intestinal epithelium during in vitro cultivation of the liver fluke does therefore seem to be appropriate. This preliminary report, giving an account of the cultivation procedures, deals only with light microscopy of the cultivated liver flukes.

Material and Methods

The liver flukes were selected from bovine bile ducts about 1 hr. after the slaughter of the animals and transferred to Hedon-Fleig or Rohrbacher solutions (*Pantelouris* 1965, p. 144) to which commercial fungicide, antibiotics and liver preparation per ml of medium were added as follows: "Mycostatin Squibb" 100 units, "Na-Penita Lääke Oy" 100 units, "Streptomycin Hoechst" 0.4 mg and "Hepavitan Lääke Oy" 0.01 ml. In all experiments 6 liver flukes were placed in a bottle containing 100 ml of one of the above described enriched media and carefully transferred to new bottles containing similar enriched medium after incubation at 37° C for 1, 2, 3, 6 and 24 hrs.' and later at 24 hrs.' intervals. The eggs produced by the flukes during the first cultivation day were counted and their capability to develop to hatched miracidia was determined.

For light microscopic studies the cultivated flukes were fixed in 10 % formalin, processed in the usual manner and stained with haematoxylin and eosin, Best's carmine and periodic acid-Schiff with and without previous diastase digestion. Fresh flukes served as controls.

Results

Continuous application of the fungicide and antibiotics appeared profitable. However, high concentrations of the mycostaticum produced slight toxicity. On the other hand, the withdrawal of the substances led to the death of cultivated flukes, due to pronounced microbial overgrowth. The application of the liver preparation appeared profitable, but no difference was found between the Hedon-Fleig and the Rohrbacher solutions.

In the ordinary experiments the flukes usually preserved a good condition and fairly active motility for about 9 days, but after that time progressively lost the normal colour and became inactive. About 16 % of the flukes remained alive for more than 12 days of cultivation, some flukes, however, survived for 15 days and 1 fluke for 18 days.

The egg production and the relative amount of hatched eggs gradually decreased. The following average values per 1 fluke as recorded during respective periods on the first cultivation day were: 3000/85 %, 2500/75 %, 2000/70 %, 1200/60 % and 100/50 %.

Excluding individual variations, glycogen in the tissues of the the flukes gradually decreased, but even the flukes cultivated for 12 days or more exhibited fairly large amounts of glycogen. The first alterations in the structure of intestinal epithelium were noted after 24 hrs.' cultivation. It was found that the height of the epithelium and the occurrence of vacuoles in the supranuclear areas of the epithelial cells did not depend on the existence of ingested fluid in the intestinal lumen. The most striking feature after that time was the increase of the vacuoles both in size and number, reaching the most frequent occurrence 3 or 4 days later. After about 1 week's cultivation the vacuoles became progressively less prominent, and various degenerative phenomena became evident both in the nuclei and in the cytoplasm of the epithelial cells.

Discussion

In previously published cultivation experiments of F. hepatica failure in the aseptic technique has been the most common cause for the short survival of the flukes (*Pantelouris* 1965). It also appears that mycostatica have not previously been used in the maintenance of liver flukes. This study shows that a con-

tinuous use of antibiotica and a fungicide is profitable and simplifies the cultivation procedures.

The origin of the vacuoles in the epithelial cells during the first cultivation days remained obscure. The vacuoles may contain accumulations of digestive secretions, but on the other hand they possibly result from pronounced pinocytosis. To elucidate the exact feature of the epithelial alterations, an electron microscopic study is necessary, for which liver flukes cultivated less than 7 days should be used.

Timo Rahko
The Department of Pathology,
College of Veterinary Medicine, Helsinki, Finland.

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