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FLUORESCENT MICROSCOPIC STUDIES ON THE MEROCYST OF THE MALARIA PARASITE

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The present paper deals with the fluorescent microscopic studies on the merocyst of *Hepatocystis* [redacted], a malaria parasite occuring in the Himalayan Flying squirrels (*Petaurista magnificus* and *P. elegans caniceps*). The induced fluorescence method of Armstrong using acridine orange for detecting nucleic acids was used, while thioflavine was used for detecting amyloid substances. It was found that the colloidal mass within the merocyst, and the outer border of the merocyst showed characteristic fluorescence for amyloids. The protoplasmic rim of the merocyst at an immature stage exhibited fluorescence for the RNA, while the merozoites occurring in the protoplasmic rim of a mature merocyst fluoresced yellow, indicating the presence of DNA.

Hepatocystis is a genus of primitive malarial parasite occurring in the arboreal mammals of the Old World tropics, as well as in the fruit bats, squirrels, flying squirrels, chevrotain and in hippopotamus. The exo-erythrocytic schizont in this genus grow in the parenchyma cells of the liver, and expand into a large structure called merocyst (10), which is visible to the naked eye. Merozoites after being discharged into the sinusoids infect erythrocytes and develop into gametocytes, which represent the only erythrocytic form of the parasite. According to earlier reports (7, 8, 11) the merocysts occur also in the spleen and the lung, besides the liver.

Structurally, a merocyst consists of a central large colloidal mass which is encircled by a peripheral protoplasmic rim studded with nuclei. Cytochemical techniques were earlier employed by one of us (3) for studying the colloidal mass and the protoplasmic rim using the merocyst of *Hepatocystis kochi*, a

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malarial parasite of the African monkey (*Cercopithecus aethiops*) as the test material.

The present study was undertaken in order to throw more light on the composition of the protoplasmic rim and the colloidal mass of the merocyst using the fluorescent microscope.

MATERIALS AND METHODS

Parts of spleen of *Petaurista magnificus* and parts of lung of *P. elegans caniceps* showing merocysts were fixed in Carnoy's fixative and cut at 4μ thickness. For the detection of nucleic acids 0.1% solution of acridine orange in neutral distilled water was used for staining the deparaffinized slides for one minute. Excess dye was washed in acetate buffer (pH 4.6) for 15 minutes. Finally the sections were mounted in fresh buffer, the edges of coverslip being sealed with molten paraffin wax.

For the purpose of studying amyloid substances thioflavine dye was used on 3μ section, 0.1% solution of the dye in neutral distilled water being employed. Deparaffinized slides were brought down to distilled water and stained in Delafield's Haematoxylin for about one and a half minutes, washed in water, placed for 10 minutes in neutral distilled water, stained in thioflavine solution for one minute, washed in distilled water and mounted in Apathy's medium, edges of the coverslip being sealed with molten paraffin wax.

The slides prepared as above were observed in Bausch and Lomb's Fluorescence microscope using blue excitor filter. For searching suitable areas in sections Y-7 density filter was used. After proper excitement of the dye photographs were taken with Kodak 35mm photographic attachment using Kodachrome's slow speed transparency (positive type) film.

RESULTS

The following observations were made :—

Acridine Orange method: In a merocyst from the spleen material the protoplasmic rim fluoresced orange red. The nuclei in the host tissue fluoresced dull yellow with a slight greenish tint (Fig. 1).

The protoplasmic rim of the merocyst from the lung material was found studded with innumerable merozoites which fluoresced yellowish orange. The

colloidal mass did not occur in the field under observation. The host cell nuclei fluoresced bright yellow (Fig. 2).

Thioflavine method : The colloidal mass and the enclosing wall of a merocyst from the spleen material fluoresced bright yellow. The protoplasmic rim of the merocyst possessed some granular substance which also fluoresced yellow, though weakly so (Fig. 3).

DISCUSSION

The central colloidal mass of *Hepatocystis kochi* is known to appear as a red homogeneous mass with Romanowsky's stain (9). According to Dasgupta (3) the colloidal mass of merocyst of the parasite stained red with pyronine, was metachromatic with toluidine blue when examined in water, and took up deep stain when Alcian blue was used. The Alcian blue-stainability remained unaffected after treatment with HCl, ribonuclease and hyaluronidase. The colloidal mass was also found to assume a faint orange colour indicating positive Millon reaction for tyrosine-containing protein. It was further observed (4) that by applying the Acridine orange method the merozoite nuclei of *Hepatocystis kochi* in advanced merocysts showed bright yellow fluorescence indicating the presence of DNA, while an early stage of the same parasite showing the characteristic infolding of the surface area fluoresced bright orange (4) indicating the presence of RNA.

In the course of the present investigation it has been noticed that the protoplasmic rim which fluoresced orange red indicated the presence of abundant amount of ribonucleic acid, while the colloidal mass was devoid of nucleic acids. RNA is known to be present in large amout during the process of growth and multiplication of the parasites as it is intimately concerned in protein synthesis. It is thus postulated that the merocyst under study was still in the stage of growth and multiplication (Fig. 1). In contrast to this the merocyst seen in Fig. 2 was in a later phase of development, the merozoite nuclei showing characteristic fluorescence for DNA, while the orange colour indicating the presence of RNA was practically lacking. The merocyst in Fig. 3 clearly demonstrates that the

colloidal mass and the wall of the merocyst fluoresced characteristically for amyloid material, as according to Culling (2) thioflavine is a specific fluorescent dye for the detection of amyloid.

The application of these dyes in the field of parasitological investigations is fruitful in many respects as pointed out earlier by one of us (6).

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Fig. 1

Fig. 2

Fig. 3

Fig. 1.: Photomicrograph of a part of section of merocyst in spleen of a flying squirrel (*P. magnificus*) infected with *Hepatocystis rayi wui*. Orange colour indicates RNA and yellow colour indicates DNA. *xca* 167.

Fig. 2.: Photomicrograph of a part of section of merocyst in lung of another flying squirrel (*P. elegans caniceps*) infected with *Hepatocystis* [illegible]. Orange colour indicates RNA, and yellow colour indicates DNA. *xca* 180.

Fig. 3.: Photomicrograph of a part of section of merocyst in spleen of *P. magnificus* infected with *H. rayi wui*. Yellow colour indicates amyloid. *xca* 200.

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ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE
LABORATORY MEETING

Liverpool School of Tropical Medicine
Thursday, 21 November, 1974
The President : PROFESSOR A. W. WOODRUFF, M. D., F. R. C. P., in the Chair.

DEMONSTRATIONS

Malaria Parasites (Hayella sp) of the Himalayan Flying Squirrel

1. Section of spleen showing merocyst
2. Section of lung showing merocyst

B. DASGUPTA AND N. L. PAL (Demonstrated by Prof. P. C. C. GARNHAM)

Hayella and *Hepatocystis* are parasites of flying squirrels and other arboreal mammals, which belong to the family Haemoproteidae as the blood stages are confined to gametocytes. Schizogony typically occurs in the form of merocyst in the liver, but in this demonstration, asexual forms were found in the spleen and the lung.

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LUNG AS A SITE OF DEVELOPMENT OF THE MALARIA PARASITE

SIR,—In the course of our recent examination of a specimen of Himalayan flying squirrel *(Petaurista magnificus)* collected at Darjeeling, we came across merocysts of a malaria parasite (tentatively identified as *Hepatocystis rayi wui* DASGUPTA et al., 1970) in the form of prominent white nodules occurring in the lung.

The occurrence of possible merocysts in the lung of a specimen of *Petaurista inornatus*, another flying squirrel, collected at Mukteswar-Kumaun, India, was earlier mentioned by the late Professor H. N. Ray (1949). It appears however that he did not examine these supposed merocysts under the microscope, and he did not preserve this material for study.

In our material the merocysts are clearly seen in the histological sections of the lung. There is a prominent colloidal mass within the merocyst. In some of the sections, islets of protoplasm occur within the colloidal mass. Besides these merocysts, some small cystic schizonts were also encountered in the lung. Further observations are in progress, and a detailed account of the findings will be published elsewhere.

We are grateful to Professor P. C. C. Garnham for his kind advice on the problem.

We are, etc.,

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