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# PRESENCE OF ANTIGENS IN CALCAREOUS CORPUSCLES OF CYSTICERCUS

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## SUMMARY

Immunofluorescence studies have been performed with histological sections of *Cysticercus cellulosae* and anticysticercus immune serum. Positive reactions revealed antigens from this parasite playing a role in the immune response of natural infections located in calcareous corpuscles of cysticercus. There are no previous records on this fact and it might be the key to obtaining more specific antigenic extracts of some helminths.

## INTRODUCTION

One of the main problems in immunology of helminthiasis is the large number of common antigens which exist in the parasites, producing cross-reactions between different helminthiasis. Some of these antigenic substances never elicit the production of antibodies in natural infections (BIAGI et al.[1]), because they do not pass from the parasite to the host but are present in the antigenic extract obtained from the parasite.

On account of both academic interest and the need of serological procedures for the diagnosis of several parenteral helminthiasis (BIAGI et al.[2]), it is desirable to have purified antigens giving specific reactions. Laboratory fractionation of raw extracts of helminths carry the risk of modifying the chemical structure of antigenic substances (KENT [4]), anatomical dissection of the parasite supplies antigens which also cross-react (OLIVER-GONZALEZ [6]). Immunofluorescency provides an opportunity to localize antigens in microscopic structures of parasites and possibility to isolate physically the microscopic structures which contain antigens. It is possible, therefore, to obtain in this way more specific antigenic extracts.

## MATERIAL AND METHODS

Living *Cysticercus cellulosae* were obtained from infected pork. Five micra histological sections of this material were prepared by standard paraffin embedding procedures (GRADWOHL [3]) with previous fixation in 10% formalin. Anticysticercus immune serum was obtained from pigs naturally infected with cysticerci. Rabbit anti-pig serum was prepared by one intraperitoneal and three intravenous injections the first 4 days of one week, and one intraperitoneal and three intravenous injections the first 4 days of the next week, of normal pig serum into rabbits; animals were bled one week after the last injection. Good anti-pig serum was labelled with fluorescein-isothiocyanate (MARSHALL et al.[5]). In order to prove the formation of antibodies against pig serum by the rabbit, the Ouchterlony's agar diffusion technique was performed (OUCHTERLONY [7]), obtaining in all cases marked precipitation lines in the agar after 24 hours.

Histological sections were deparaffined, rehydrated, covered with anticysticercus pig serum and left at 37°C for 30 minutes. They were then washed, covered with fluorescein-

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labelled anti-pig rabbit serum and left at 37°C for 30 minutes. To counterstain, bovine albumin labelled with rhodamine was used (SMITH et al.[8]). Finally, the sections were washed, a coverslide was placed over glycerine, and observed in a standard fluorescency microscope.

Control slides were prepared using sera from pigs without cysticercosis. Natural fluorescency of the parasite was checked in unstained sections. Slides were also prepared with only the rhodamine albumin counterstain.

## RESULTS AND COMMENTS

Throughout this procedure it was surprising to find that most of the fluorescein appears in calcareous corpuscles, which were completely green when anticysticercus sera were used (Fig. 1) and were non-fluorescent in sections treated with normal pig sera (Fig. 2). Less fluorescein appears in the membrane or in other places of scolex of the *Cysticercus* and none in the cuticle. Rhodamine counterstain was the only one present in the cuticle, was also distributed in the parasite's body and absent in calcareous corpuscles.

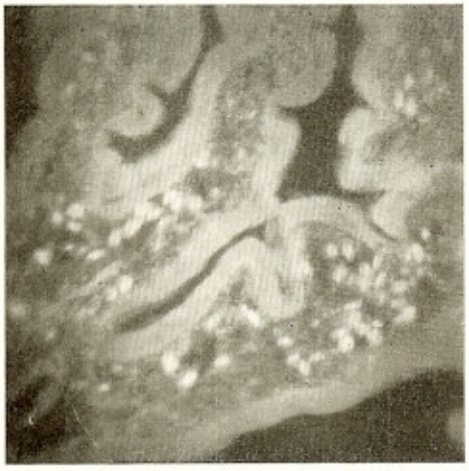

Fig. 1 — Bright calcareous corpuscles (oval shaped) because of green fluorescency in a histological section of *Cysticercus cellulosae* treated with anticysticercus sera.

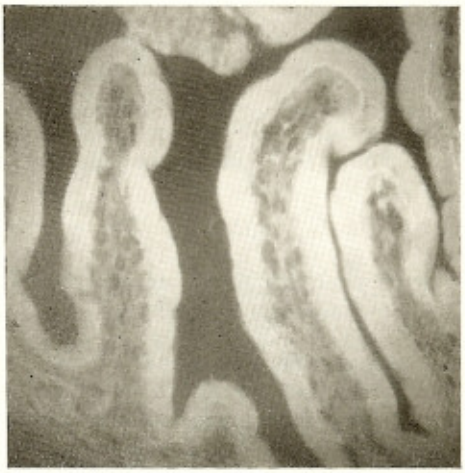

Fig. 2 — Dark calcareous corpuscles (non-fluorescent) in a section treated with non-immune sera.

## DISCUSSION

Considering that anticysticercus sera from natural infections were used, the presence of fluorescein is interpreted as present in antigenic substances which play a role in the immune response during a natural infection; then, calcareous corpuscles appear as microscopic structures having a larger quantity of antigens. These findings were confirmed in several instances with sera from different pigs. Natural fluorescency (green) was observed only in the hooks.

Knowledge of calcareous corpuscles is meagre; they disappear during incubation in non-nutritive media, and may have a role as a buffer against extraneous acids (VON BRAND [9]). Possibly some of the proteins and polysaccharides of the calcareous corpuscles leave the parasite during its metabolism and pass to the host to elicit the production of antibodies.

This finding is important in order to prepare better antigenic extracts because it is very easy to separate calcareous corpuscles from other structures of the parasite and in this way the antigenic extract will be less contaminated with substances from other places of the parasite which do not play a role in the immune response in the natural infection. Knowing calcareous corpuscles'

specific functions, it is possible that their chemical composition will be of less complex substances than the whole parasite. It is possible, therefore, that the number of unspecific antigens will be less in extracts obtained from calcareous corpuscles.

RESUMO

*Presença de antígenos nos corpúsculos calcários do cisticerco.*

Estudos de imunofluorescência foram feitos em cortes histológicos de *Cysticercus cellulosae* com sôro imune anticisticerco. As reações positivas revelaram a presença de antígenos dêste parasito, desenvolvendo uma função na resposta imunológica às infecções naturais, localizada nos corpúsculos calcários do cisticerco. Não há registro anterior sôbre êste fato que pode ser a chave para a obtenção de extratos antigênicos mais específicos, de alguns helmintos.

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