

**ACTINOMYCES BOVIS A POSSIBLE CAUSE FOR CALCIFICATION
OF THE PARASITE IN THE HOST TISSUE***

by

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Abstract

Strains of *Actinomyces bovis* were isolated from calcified granules in the lesions bordering hydatid cyst and cysticercus bovis, and also from aspirate and homogenized cysticercus bovis.

It is believed that the *Actinomyces bovis* might be responsible for degeneration and calcification of the cysticercus bovis and hydatid cyst.

It deposits in the tissue bordering the parasite and invades into the parasite when the lesion becomes chronic.

Introduction

Smither et al 1,2,3 to account for their observation on the continued survival of *Schistosoma mansoni* in the hosts, postulates that migrating parasites become coated with certain contaminating host antigens which thereby interfere with the immunological reaction and enhance parasitic survival.

Brown and Tanner,4 indicated long lag phase (60 days) in mice after inoculation of single cyst of *Echinococcus multilocularis* to start to grow and they mentioned "Immune surveillance" could be the mechanism which controls cyst growth during this early stage of the infection.

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Sarajam⁵ after experimental infection of mice with *Echinococcus multilocularis* described acute granulomatous inflammation which later becomes chronic.

Study of Brown and Tanner⁴ on thymectomized mice showed that cell mediated immunity cannot control the dissemination of *Echinococcus multilocularis* during the early phase of infection. Varela -Diaz and Coltorti, 6,7 demonstrated the presence of host immunoglobulins in hydatid fluid and cyst membrane of *Echinococcus granulosus*. Chris⁸ after study on the infected mice with *Taenia crassiceps* believed that the antibodies produced by the host are not effective against parasites.

In the present study, after repeated isolation of *Actinomyces bovis* from granuloma around the cyst and cysticerci also from the inside of some *cysticercus bovis*, the suspicion arises that this microorganism is responsible for calcification.

Material and Method

Calcified granules were obtained from lesions beneath *cysticercus bovis* in the heart muscle and also from sterile hydatid cyst in the liver of slaughtered cattle in the Teheran abattoir.

Granules were washed with sterilized distilled water, homogenized and cultured in brain and heart infusion agar, (Difco), Sabourand's maltose agar (Difco), thioglycollate broth (Difco) and *Actinomyces* broth (BBL).

Aspirated fluid and homogenized cysticercus (removed from the heart muscle of a cow) were cultured in the same way. All cultures were kept under anaerobic conditions for two weeks. The basic medium for fermentation tests consisted of sodium thioglycollate in pH 7.2, with added Bromo thymol blue (12%) to detect acid formation. The desired carbohydrate was added to give a final concentration of 1.0%.

Gas production was determined by durham fermentation tubes and readings were made after 20, 25-30 days. Catalase production was determined by adding a drop of 3% H₂O₂ to colonies on BHI plates or by adding a drop of 3% H₂O₂ to heavy suspension on a microscope slide.

Gelatin, MR-VP (BBL) medium triple sugar iron (TSI) agar , nitrate agar, triptophane broth, Simmon's citrate agar, were used for gelatin liquefaction , Vosges-Proskauer and methyl-red reaction, H₂S production, nitrite, indole and citrate fromation tests, respectively.

Results

Six smooth and two rough strains were isolated from calcified granules, situated in the lesions bordering hydatid cyst and *cysticercus bovis*, aspirated fluid form *cysticercus bovis* and homogenized whole *cysticercus bovis*.

Colonies were obligate anearobe, white, raised, soft, or adherent to medium, smooth and rough, and had a good growth on brain heart infusion agar

(BHI) and thioglycollate broth with indicator, under complete anaerobic condition.

Smooth colonies in tubes with liquid medium settled to the bottom of tubes, when the contents of the tubes were swirled, spiral forming was observed (Fig. 1). While rough colonies in thioglycollate broth formed bread crumb colonies like those described in *Actinomyces israelii* 9 (Fig. 2,3).

On BHI, rough colonies adhered to the medium with branched mycelia (Fig. 4). The organism was gram positive, non motile non sporolate.

Smear from smooth colonies showed diphtheroid forms, with varying lengths. (Fig. 5). Knobbed ends V and Y shapes were also observed .

Branched mycelia were observed in smears from rough colonies (Fig. 6).

Inulin, fructose, galactose, glucose were fermented by all strains, while arabinose was variable. None of the isolated strains fermented Raffinose, dulcitol, salicin, dextrin. No gas was produced. Except for one (VM6), all smooth strains were catalase negative. All were urease and indole negative. All smooth strains liquefy gelatin. All strains were VP test negative and MR test positive. Table I shows the results of biochemical tests.

Discussion

Possibly “ Immune Surveillance” mentioned by Brown and Tanner⁴ is a critical stage when a parasite begins to be established in the host tissue. An antigenically inactive parasite could act like a foreign body and be subjected to accumulation of microorganisms like *Actinomyces*, which has been isolated from the blood of a human host.¹⁰

Routine observations of the parasite in the host tissue indicate -that the successful parasite is the one that is able to manipulate host reaction to form a classical granulomatous cavity and lie safely in it.

The role of extremely dialated vascular reaction which occurs in this stage around the parasite (due to the observation of Sarajan⁵) seems also to be important.

The observation of host immunoglobulin being passed through the cyst,^{6,7} indicates the inter-relation between parasite and host possibly through the blood circulation neighbouring the parasite. Such circulation bordering parasitized area might also be-helpful in reducing the number of micro-organism deposits and which super infect the tissue.

Especially they are the basis for leucocytic infiltration which could phagocytize the microorganism.

As far as parasite stimulates the host reaction or the host responds to stimulation, the parasite continues to live.

Due to the observation of Sarajam, 5 in the later stage with chronic granulomatous inflammation excessive fibrosis of tissue cuts down the blood supply and consequently the tissue undergoes degeneration. Obviously reduced blood supply in this stage encourages the growth of microorganisms in this area.

The present study indicates that established colonies of *Actinomyces* in parasitized and blood reduced areas (chronic type) gradually invade the cysticercus or other metacestode in the host tissue, and cause them to degenerate and calcify.

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Table 1

Tests	Strains							
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	R ₁	R ₂
Catalase	0	0	0	0	V	0	V	V
Nitrate reduction	+	+	+	+	+	+	+	+
Indol	0	0	0	0	0	0	0	0
Litmus milk	A	A	A	A	V	+	V	V
Gelatin liquefaction	+	+	+	V	V	+	V	V
Urease production	0	0	0	0	0	0	0	0
SH ₂ Production	0	0	0	0	0	0	0	0
Inulin	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Arabinose	V	V	V	V	V	V	+	V
Raffinose	0	0	0	0	0	0	0	0
Dulcitol	0	0	0	0	0	0	0	0
Dextrin	0	0	0	0	0	0	0	0

+ Acid produced in 1 to 25 days.

0 No acid produced.

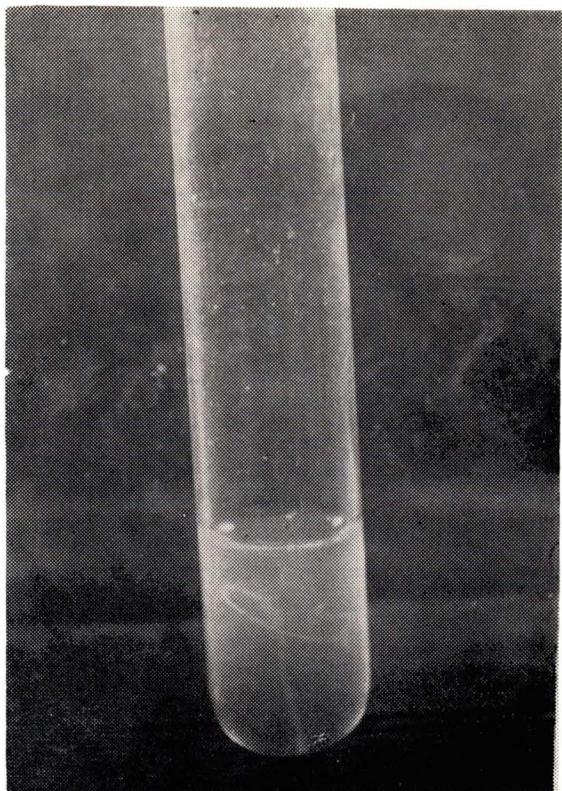


Fig. 1. Spiral forming of the smooth colonies in liquid medium.

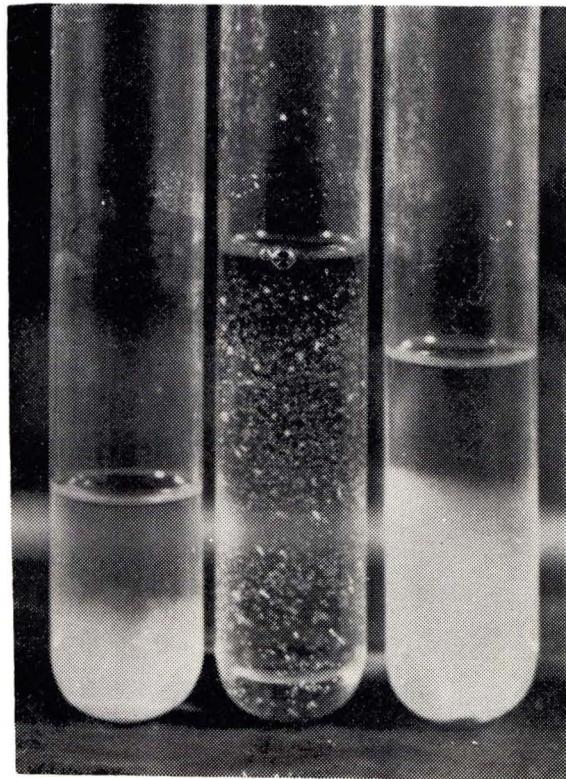


Fig. 2. Bread Crumb formig (Rough Colony) in broth

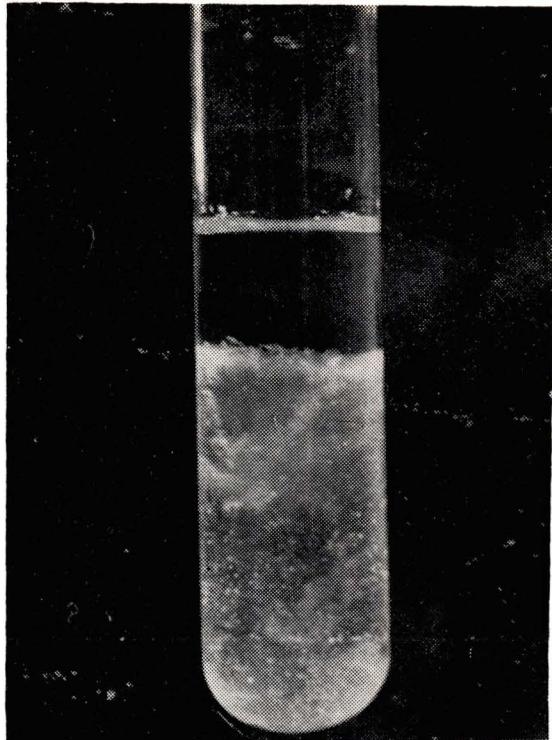


Fig. 3. in thioglycollate



Fig. 4. Rough colonies on BHI Medium



Fig 5. Smear from smooth colonies X 400



Fig. 6. Smear from rough colonies X 1000



Fig. 7. Smooth colonies on (BHI) Medium