

SEATO Medical Research Study on Amebiasis

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Introduction

The object of this study is to identify factors involved in the causation of amebic disease. There is strong evidence, derived from different experimental approaches to suggest that E. histolytica alone is non-pathogenic. If this is correct, the etiology of amebic disease requires explanation. This study has been designed to investigate the possible influence of viral and toxic agents on the causation of amebic disease. This has been approached in two ways: First by the processing of material from cases of human liver abscess for the isolation of a virus component. The second approach has been experimental. This has compared, in animals, the effect of inoculation of cultures of E. histolytica: (1) with and without selected viruses and (2) in normal and intoxicated animals. Amebic cultures have been either bacteria-free or grown with a single bacterium which could be inhibited by antibiotics for pathogenicity trials. The relationship between E. histolytica and the viruses employed was also investigated.

Conclusions

A) Concerning the Pathogenicity of E. histolytica and the Effect of Possible Potentiating Factors.

1. Bacteria-free preparations of the dysentery ameba, E. histolytica have proved non-pathogenic and incapable of survival. These preparations include cultures of two strains and material from human liver abscess. Doses were in the 10^3 range and did not exceed 4×10^3
2. Animals systems and routes employed include: (1) the embryonated chicken egg inoculated on the chorio-allantoic membrane, in the chorio-allantoic fluid and in the yolk sac (2) Young mice inoculated intra-cerebrally (3) Hamsters inoculated intra-hepatically and intraabdominally.
3. E. histolytica strain 301 could be maintained in culture by indefinite transfer and produced high yields when grown in mono-bacterial culture with Clostridium perfringens. These cultures were tested in hamsters, after bacterial inhibition with antibiotics both in vitro and in vivo.
4. Mono-bacterial antibiotic-inhibited cultures of strain 301 produced liver abscesses in hamsters at doses of 3 to 5×10^5 when inoculated intra-abdominally or intra-hepatically. Liver abscess was never produced at doses below 1×10^5 . Accordingly the effect of added factors was investigated by using non-pathogenic doses and selected added factors which included viruses and hepato-toxic chemicals.
5. The viruses tested were Herpesvirus hominis and Coxsackie strains A9, B5 and B6. These were inoculated intra-hepatically, occasionally intra-abdominally at doses known to produce lesions in tissue cultures and/or animals. Virus inoculations were followed by injection of non-pathogenic doses of strain 301 into the liver or the abdominal cavity. In general either no potentiating effect was observed (herpesvirus, Coxsackie A 9 and B 5) or the effect was a marginal one (Coxsackie B6).

6. The chemical, toxic for the liver, which was chosen was carbon tetrachloride given intra-abdominally at or near maximum tolerated dosage. This was followed in 24 hours by intra-hepatic inoculation of non-pathogenic doses of strain 301 and a strain (PT) newly isolated from a case of liver abscess in Bangkok. With both strains, intoxicated animals developed liver abscesses; controls were negative. Liver abscess production was approximately 25% (of 29 experimental animals) with no liver abscesses in 27 controls.
7. The overall conclusion from this phase of the work is that the essential non-pathogenicity of E. histolytica is confirmed for the strains here utilized. Pathogenicity was either not increased by viral-amebic association or the effect was marginal. Pathogenicity was increased in carbon tetrachloride intoxicated hamsters, liver abscesses developing with doses of E. histolytica which were consistently non-pathogenic in normal animals.

In evaluating these results, it should be noted that the hamster, although the most satisfactory laboratory animal found, does not provide a particularly sensitive test system: monobacterial cultures grow out regularly from an inoculation of 10 amebae, whereas monobacterial cultures of E. histolytica (301) are not recovered from the hamster liver until the inoculum reaches 2 to 5×10^5 organisms.

B) Virus Isolation Trials from Liver Abscess Materials

- 1) Amebic liver abscess material is usually bacteriologically sterile. Reports of investigation of such material for viruses have not been found in the literature. Successful virus isolation would depend on long persisting virus occurring either in host tissue or in association with amebae.
- 2) Twenty-eight aspirates from as many cases of human liver abscess were processed in cooperation with the Department of Virology. Aspirate samples were inoculated into two types of tissue cultures (MK and Hep 2) and into infant mice by the intra-cerebral and intra-peritoneal routes.
- 3) No viruses were isolated, indicating that viruses detectable by the testing systems were absent. No information is provided on the possible role of non-persisting viruses at the time of initial seeding of the liver with amebae.

C) Relationship of E. histolytica to viruses.

- 1) If viruses and amebae initiate liver lesions, then a transport mechanism where by the two agents arrived in the same liver area together would clarify their coincident action. Accordingly the survival of viruses with amebae was investigated.
- 2) Amebae were washed in tissue culture medium, exposed to herpesvirus, Coxsackie A 9, B 5 and B 6, incubated at 37°C. and returned to cultures. At intervals of 24 and 48 hours washed amebae were tested for virus, dilution of the original virus inoculum approaching the limits of sensitivity of the testing system used.
- 3) Herpesvirus was not detectable after 24 hours, and Coxsackie B 6 was not recovered after 48 hours. Coxsackie strains A 9 and most B 5 preparations brought down all tissue culture tubes after 48 hours. This proves virus survival for this period and suggests a possible transport mechanism.
- 4) The additional question of viral multiplication in amebic cultures is being investigated in experiments which have not yet terminated.

D) Preservation of cultures at -85°C.

- 1) Cultures of E. histolytica were preserved for three months frozen and stored at -85°C. Slow freezing appeared preferable to fast; a suspension in 5% dimethyl sulfoxide superior to 5% glycerine. There appeared to be deterioration at -85°C, as no specimens stored six months or longer were viable. Storage in liquid nitrogen may permit prolonged survival. Differences in strains were observed; using apparently identical methods one recently isolated strain proved almost totally refractory to frozen storage.