

Comparative Pathophysiology of Strains of E. histolytica

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OBJECTIVE: To investigate the comparative invasive traits of strains of E. histolytica in SE Asia to determine whether differences in proteolytic enzyme activity accounts for some strains colonizing in the liver rather than producing the classical colonic ulceration with typical amebic dysentery.

DESCRIPTION: As previously reported (Annual Report 1970-1971) enzymes studied are aminopeptidase using glycine as a standard; dipeptidase using glycylglycine as a standard; pepsin; trypsin; carboxypeptidase; hyaluronidase; hydrolase using casein, hemoglobin and gelatin as substrates. Axenic strains of amebae are cultivated in a monophasic media which permits harvesting of relatively clean populations. Attempts are made to culture amebae from colonic lesions, amebic abscesses and from cyst passers without symptoms.

PROGRESS: Several attempts to adapt isolates of E. histolytica to the monophasic media have been unsuccessful, and enzymatic studies have been restricted to the axenic strains HK-9, HLT10 and HLT12. HK-9 is a strain isolated by W.W. Frye from proctoscopic material several years ago and is common culture to many laboratories. HLT10 was isolated directly from the pus of a liver abscess in August, 1970, by Dr. L.T. Wang from a patient in Taiwan who suffered from dysentery as well as multiple liver abscesses. HLT12 was also isolated by Wang in November of the same year from a liver abscess. The homogenates of these strains were prepared by washing the parasites 3 times in physiological saline, counting them in a hemacytometer and then homogenizing them in an ice bath. Following centrifugation at 12,000 rpm for 10 minutes the supernate was used for the enzyme assays. The activity of enzymes was measured by spectrophotometric analysis.

The enzyme activities determined for these three strains differed only in the hyaluronidase activity. As shown in Table 1, this enzyme was detected to be of low level activity in strains HK-9 and HLT10, and was absent in HLT12.

The World Health Organization Expert Committee report No. 421 suggests that amebae cultured in vitro in the presence of cholesterol, or if the hosts are given high doses of this steroid by mouth, it is possible to convert non-invasive strains isolated from asymptomatic human subjects into invasive strains indistinguishable from those isolated from patients with amebic dysentery. To test this, the strains cultured in this laboratory were placed in media containing 0.05 mg cholesterol per ml. When analyzed for enzymatic activity, the only difference detected was in the hyaluronidase activity (Table 1). Strain HK-9 advanced from weak activity (\pm) to strong positive (+), and where it had been absent in strain HLT12 the enzyme was readily detected after the introduction of the cholesterol.

Hamsters 4 weeks old were inoculated directly into the liver with the HK-9 strain cultured both in the presence and absence of cholesterol. After two weeks the animals were examined for abscess formation. No lesions were present in those hamsters which had received the amebae not exposed to cholesterol, and one abscess was found in one hamster which received the culture with cholesterol. Microscopic examination of this abscess revealed E. histolytica. The numbers of hamsters were small (4 in each group) and no significant conclusions can be drawn from this attempt. Further studies to elucidate this suggested enhancement of pathogenicity are in progress.

SUMMARY: Enzyme analyses of three strains of axenically cultured E. histolytica showed differences only in hyaluronidase activity. This enzymatic activity was enhanced in two strains by culturing the amebae in the presence of cholesterol. An amebic abscess was produced in a hamster after exposing the amebae to cholesterol.

Table 1.
Enzyme Activity of Axenic E. histolytica Cultures

Proteolytic Enzyme	Cultivated in Diamond's Media			Cultivated in Diamond's Media with Cholesterol		
	HK-9	HLT 10	HLT 12	HK-9	HLT 10	HLT 12
Aminopeptidase	+	+	+	+	+	+
Dipeptidase	+	+	+	+	+	+
Hyaluronidase	±	±	-	+	±	+
Pepsin	+	+	+	+	+	+
Trypsin	+	+	+	+	+	+
Carboxypeptidase	+	+	+	+	+	+
Hydrolase:						
Casein	+	+	+	+	+	+
Haemoglobin	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+