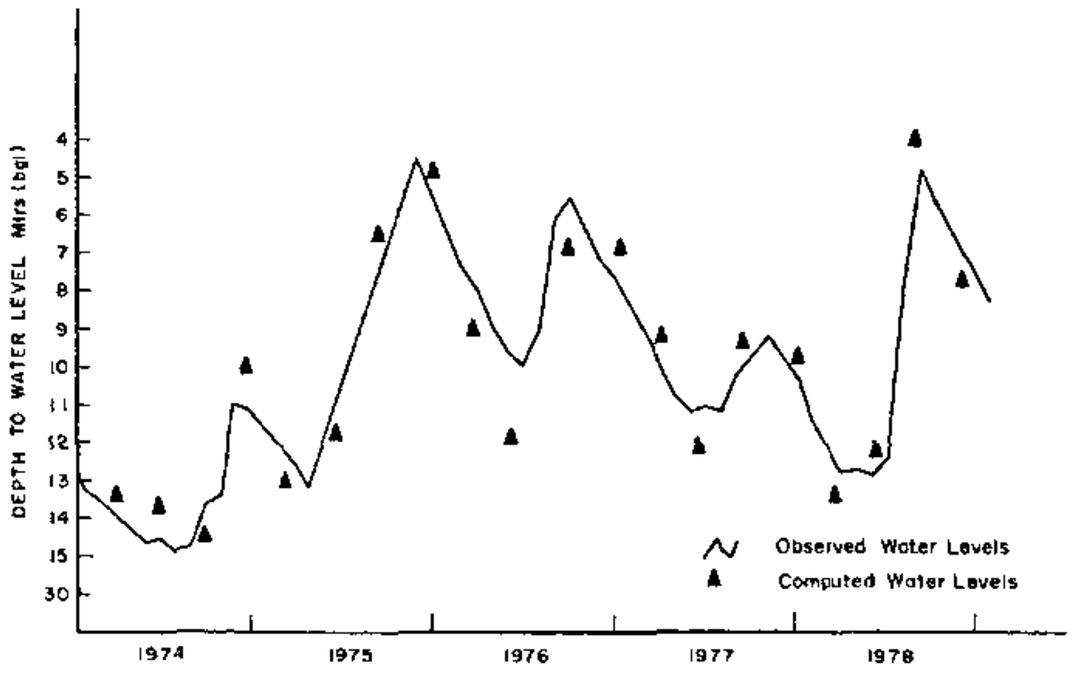

Figure 2. Observed and computed water levels at Shadnagar.

November of each year are entered into the computer to calculate the water levels during these months. As an example, the computed water levels at Shadnagar are compared with the actual hydrographs for this well for the years 1974–78 (figure 2). From this figure and from the data of other well sites, it could be seen that the computed values agree well with the hydrographs. Deviations from the actual water levels to an extent of 1.5 m are noticed at a few points but these are to be expected. In particular the oscillating character of the curve during each of these years is faithfully followed by the computed water levels. Error accumulation is also studied during this period of simulation and is found to be insignificant as the total change varied from 0.1 to less than 0.5 m.

Conclusions

The calibrated digital model is utilized to study the sensitivity of the aquifer parameters by carrying out several experimental runs. It is observed that water levels are sensitive to transmissivity variations and hence have to be carefully determined especially in low transmissivity regions. Storativity has direct and proportionate bearing on the net recharge distributions. A special effort should be made to accurately determine these parameters for the right assessment of hydrogeological condition of the basin. The confidence in the model is also assured by the faithful simulation of long duration hydrographs over a period of five years.

Acknowledgements

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ELECTROPHORETIC STUDIES OF THE HEXOKINASE OF *ENTAMOEBA HISTOLYTICA*

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Abstract

Cellulose-acetate gel electrophoretic studies on the hexokinase of classic *Entamoeba histolytica* strains, DKB, IP-106 and NIH-200 and the clonal cultures developed from these strains showed significant variations in the mobility of the isoenzyme in the parent cultures and their clones. The parent strains of *E. histolytica* consist of a mixture of amoebae in the cultures.

Introcuction

INTESTINAL amoebae of a man have been characterized and grouped by their isoenzyme patterns[1-4]. Cloning of *E. histolytica* cultures is essential to confirm if the cultures contained mixture of amoebae[4]. A method for successful clonal growth of axenically grown *E. histolytica* was reported[5]. Electrophoretic study[4] of hexokinase of *E. histolytica* group I to IV clearly identified group II from other three groups by the advanced position of bands. Group II amoebae were associated with clinical amoebiasis.

The isoenzyme studies of *E. histolytica* so far are carried out with the cultures which did not develop from a single trophozoites or cyst; it is possible that a mixture of amoebae might be present in the cultures.

We report here the result on hexokinase of *E. histolytica* in the parent cultures, and clonal cultures developed from the parent cultures.

Materials And Methods

Strains of *E. histolytica*, DKB, IP-106 and NIH-200 used in this study, were brought from Prof. E. Meerovitch, Institute of Parasitology, Canada by one of the authors (SRD). The first strain had lost its pathogenicity after prolonged *in vitro* cultivation. The last tow strains are *pathogenic to rat caecaum and hamster liver*[6]. Clonal cultures of these strains were developed following the method of Das and Meerovitch[5]. Perspex-perfusion chambar[7] was used for clonal growth of amoebae. Single trophozoite, from a drop of amoebic suspension on a sterile slide, was picked up by a microcapillary, under the microscope inside a laminar flow hood, and transferred to the cavity of the chamber containing TYI-S-33 axenic medium[8]. Both parent cultures and clonal cultures are maintained in this medium.

Preparation of lysates and electrophoresis were carried out[1] and the cells were ruptured by ultrasonication at 10 amplitude for 5 minutes at 0° C instead of freeze-thawing. Lysates were subjected to electrophoresis using CELLOGEL-500 (CHEMITRON G MODENA 24, 20129 MILLANO) at 100 volts for 2 hr. The electrode buffer consisted of 0.1 M Trismaleate with EDTA and magnesium chloride pH 7.4 (12.18 g, Tris; 11.61 g, maleic acid, 2.03 g, $MgCl_2$; 3.73 g EDTA, 12.18 ml. 10 N NaOH, H_2O to one litre). After eletrophoresis the enzyme was visualised by formazan development in agar overlay using the method of Farri *et al*[3].

Results

Figure 1 is the hexokinase zymogram of parent cultures of *E. histolytica* DKB and NIH-200 and their clonal cultures, showing the band position (i) bacterial control, (ii) parent DKB strain, (iii) clone of DKB, (iv) parent NIH-200 strain (v) clone no. 1 of NIH-200, (vi) clone no. 2 of NIH-200 (vii) clone no. 3 of NIH-200. Figure 2 is the hexokinase zymogram of parent IP-106 strain of *E. histolytica* and its clonal cultures showing the band position (i) bacterial control, (ii) parent IP-106 strain, (iii) clone no. 1 of IP-106, (iv) clone no. 2 of IP-106, (v) clone no. 3 of IP-106 and (vi) clone no. 4 of IP-106. Figure 3 is the diagramatic representaion of band position for parent cultures and the clonal cultures.

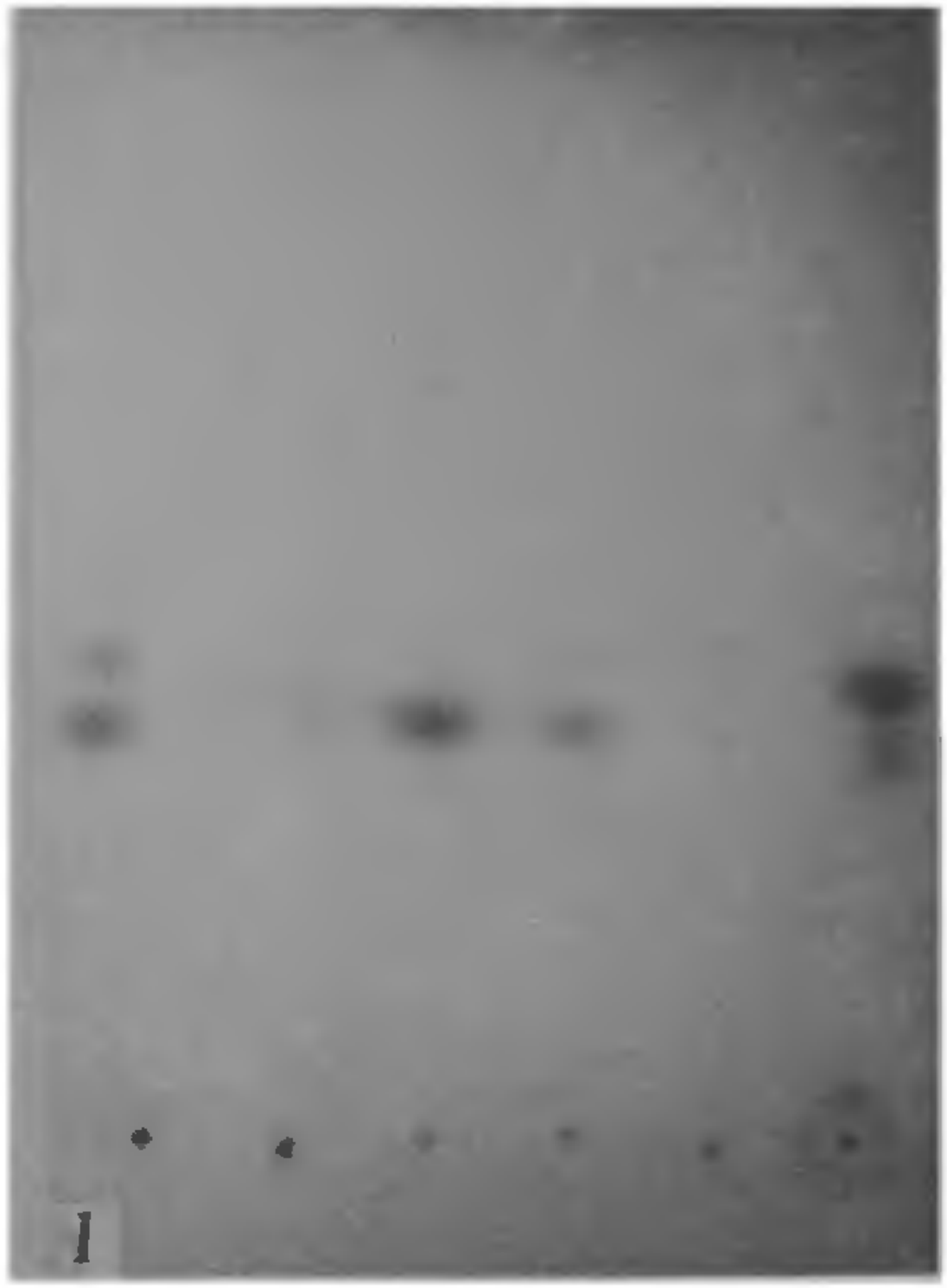

Figure 1. Showing hexokinase xymogram of parent cultures of *E. histolytica* DKB and NIH-200 and their clones with bacterial control.

It is interesting to observe that the hexokinase zymogram of parent strains of *E. histolytica* IP-106 and NIH-200 showed very little variations in the position of bands. These two strains of *E. histolytica* are pathogenic to host tissues. However, the non-invasive DKB strain of *E. histolytica* showed variation in the position of band, which is below the band positions of IP-106 and NIH-200. Clone no.1 of DKB and parent strain NIH-200 showed similar band positions, which were little above the parent DKB strain. All the three clone cultures of NIH-200 strain of *E. histolytica* showed variation in their band position among themselves as also from the parent strain. Pathogenic IP-106 strain of *E. histolytica* showed the highest mobility of isoenzyme as compared to others. Clone no. 1, 3 and 4 of this strain showed exactly similar band positions whereas clone no. 2 showed marked difference in band position (figure 3). The position of bands of isoenzyme of DKB clone no. 1, parent strain NIH-200, clone Nos. 1, 3 and 4 of strain IP-106 showed similar band positions. Similar band positions were observed in the parent strain DKB, clone no. 2 of NIH-200 and

Figure 2. Showing hexokinase xymogram of parent culture of *E. histolytica* IP-106 and its four clones with bacterial control.

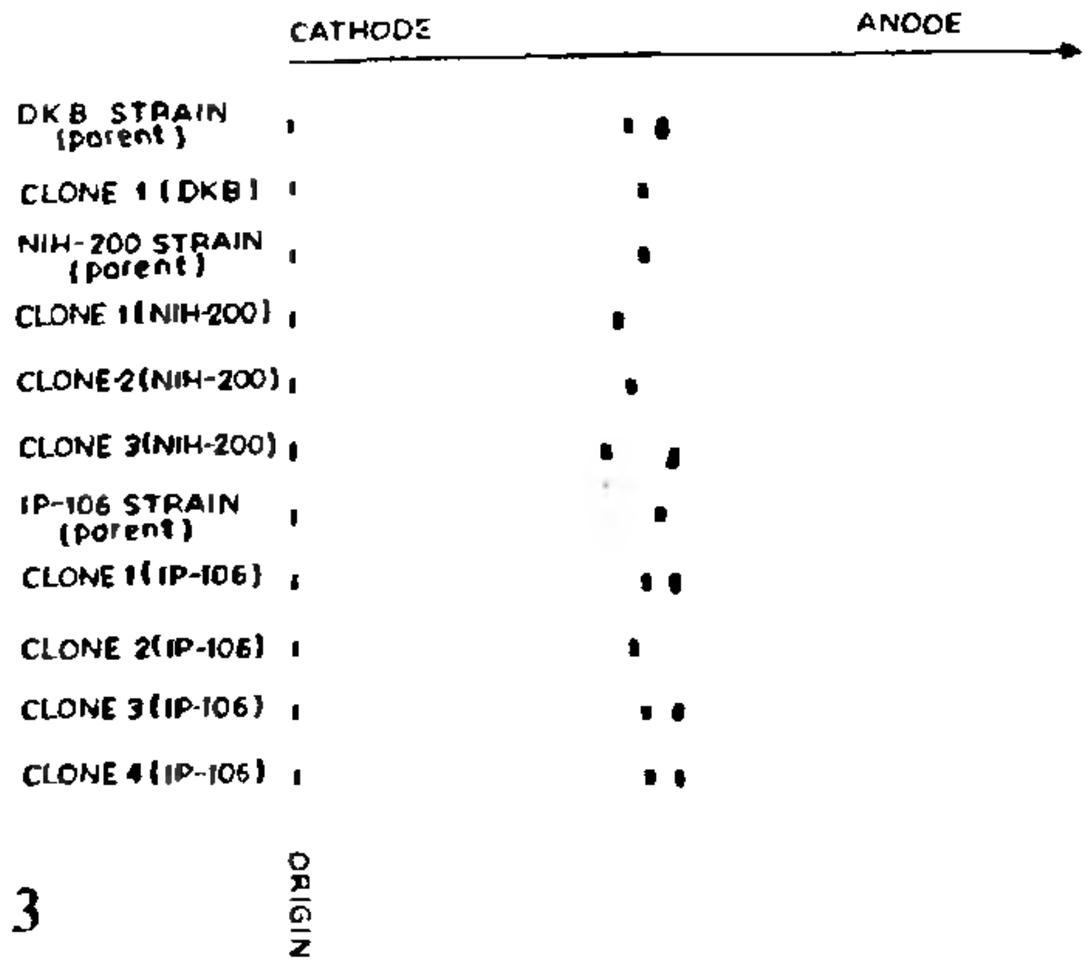

Figure 3. Diagramatic representation of the band positions for the parent cultures and the clonal cultures of *E. histolytica.*

clone no. 2 of IP-106. Marked difference in band position was observed in clone no. 3 of NIH-200 strain of *E. histolytica* (figure 3).

It is, therefore, suggested that amoebae in clones DKB no. 1, IP-106 no. 1, 2 and 3 are similar. Amoebae of clone no. 2 of NIH-200 and clone no. 2 of IP-106 are also similar. All the parent strain DKB, IP-106 and NIH-200 do not consist of only one type of amoebae; on the other hand, they consist of a mixture of amoebae. Virulent IP-106, strain of *E. histolytica* could easily be differentiated from the other strains by isoenzyme electrophoresis. Clonal growth of *E. histolytica* cultures are essential to study on various aspects of this amoebae such as cell biology, behaviour, pathogenicity, antigen preparation and so on.

Discussion

Enzyme polymorphism in *E. histolytica* has been reported earlier[2]. Differentiation between invasive and non-invasive strains of *E. histolytica* is also possible by isoenzyme electrophoresis[2]. Isoenzyme study of *E. histolytica*[2-4] is a valuable marker for grouping of *E. histolytica* strains. Isenzyme studies so far carried out are with strains of *E. histolytica* growing in association with bacteria. No report is available on isoenzyme studies of *E. histolytica* with clonal cultures of exenically or polyexenically grown E. histolytica. Nothing so far is known if the parent strains of *E. histolytica* consisted of mixture of amoebae in the cultures. This is the first report on isoenzyme eletrophoretic study of hexokinase of clonal cultures of *E. histolytica.* The present result reveal that the three classical strains of *E. histolytica* DKB, IP-106 and NIH-200, consist of a mixture of amoebae in the cultures.

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EFFECT OF DIETHYL CARBAMAZINE CITRATE ON PRECOCIOUS PUBERTY INDUCED BY ESTRADIOL MONOBENZOATE IN IMMATURE RATS

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Abstract

Precocious puberty along with superovulation induced by administering exogenous estradiol monobenzoate in immature rats was totally blocked by concommitant administration of diethyl carbamazine citrate. This suggests that diethyl carbamazine citrate significantly blocks the central positive feedback effect of estrogen on the release of gonadotrophin or hypothalamic gonadotrophin releasing factors.

Introduction

SOME of the piperazine derivatives exhibit varying degrees of antifertility effects[1]. The mechanism of action of diethyl carbamazine citrate (DEC) as an oral contraceptive has not been studied. An extensive study was therefore undertaken to explore the possible mechanism of action of DEC as an antifertility agent in albino rats[2].

Induction of precocious puberty along with superovulation by exogenous administration of small doses of estrogen is chosen as a parameter because it can be specifically blocked by an anti-gonadotrophic agent. This paper presents the effect of DEC on superovulation in immature rats.

Materials and Methods

Immature (27-day old) female albino rats (Wistar strain), inbred in the departmental animal house and weighing between 35–40 g were used in the present study. The rats were maintained under controlled light (12 hr light:12 hr dark) and constant temperature (22 ± 2° C) and fed with standard pellet diet (Hindustan Lever) and tap water *ad libitum.*

The drugs and vehicle were administered between 12.00 and 13.00 hr everyday to avoid diurnal variation. DEC was dissolved in distilled water and the volume was kept constant at 0.1 ml per animal irrespective of dose and route of administration. Estradiol monobenzoate (EM) was suspended in sesame oil and the volume was kept constant at 0.1 ml per animal. All the rats were divided into different subgroups. Each subgroup consisted of 10 animals. The rats were treated for 3 days as shown in table 1. On the 4th day, all the animals were sacrificed by cervical dislocation, their body wights were recorded and the vaginal state was observed (if opened, smears were taken and scored after methylene blue staining). Their uteri were quickly removed, free of fat and connective tissue and their intraluminal fluid expressed by pressing between filter paper foldings. The tissues were weighed on a torsion balance.

In animals with vaginal opening, vaginal smears were taken and stained with methylene blue for 10 min and scored under microscope[3]. The oviducts of these animals were carefully separated from the ovaries and the ova within them were expelled by flushing with normal saline. The ova were counted under dissecting microscope. Evans blue was used as a vital stain after being dissolved in hyaluronidase (1%).

The ovaries were fixed in 10% formaline saline, serially sectioned at 6 μ and stained with haematoxylin and eosin and screened.

Results

The wet weight of uterus, the percentage of vaginal opening and the number of eggs per rat are given in table 1. In the control and DEC-treated rats there was no vaginal opening. Their uterine weights were significantly low when compared to that of estrogen-treated group. The vaginae of the animals treated with EM opened on the 4th day of the treatment; the vaginal smears showed plenty of cornified epithelial cells in layers (estrus). The number of ova released during this superovulatory period was counted (mean 10.20). The uterine weights were significantly increased when compared with vehicle-treated control group ($p < 0.001$). In the rats treated with EM μ DEC combination (irrespective of route of administration of DEC), there was a significant increase in the uterine weights with no sign of ovulation and the vaginae remained closed.