provide evidence of the collagenous nature of the protein in question, based on quantitative estimation of glycine. In this context it is of interest to recall the observations of RUDALL[14] that the protein of the soft cuticles of lobsters and insects show chemical features reminiscent of collagenous proteins.

Zusammenfassung. Der einzige Proteinbestandteil des Branchiopodenkrebses *Streptocephalus dichotomus* zeigt ähnliche chemische Eigenschaften wie kollagene Eiweissverbindungen. Merkmal der Aminosäurezusammensetzung ist eine relativ grosse Menge von Glyzin (etwa $^1/_3$ des Gesamtresiduums), zusätzlich zu Hydroxyprolin und Prolin. Dies wird als Kriterium zur Unterscheidung von kollagenem Protein von den übrigen Eiweissen (K-mef-Gruppe) benützt.

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University of Madras, Zoological Research Laboratory, Madras 5 (India), 19 November 1968.

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Division of *Entamoeba invadens* Rodhain, 1934, in Cultures[1]

The nuclear division of *Entamoeba* has been studied by several authors. The Table summarizes these observations. So far, very little attention has been paid to the cytokinesis of *Entamoeba*. The term 'cytokinesis' has been used here as defined by MAZIA[2] which includes all the schemes of cytoplasmic division during the process of mitosis.

From the survey of the literature on the cell division of *Entamoeba*, it appears that HARRIS[3] first saw the cytoplasmic division of a trophozoite of *E. histolytica* (Amoeba dysenteriae) in the faecal smears. He observed the trophozoite elongated to many times its width, and one end moved more rapidly, creating a constriction near the middle. The daughter amoebae remained connected for a while by a long but less that 1 μ wide 'band, composed entirely of ectosarc'. Usually, division into 2 was observed by HARRIS but once he saw a division into 3 (his Figure 1d, p. 568). DOBELL[4] considered that HARRIS observed a pathological process, because it was studied at room temperature. DOBELL[4] has argued that the sudden change of temperature might have caused such division referred to above in *E. histolytica* trophozoites.

SHAFFER et al.[27] have studied the cytokinesis of *E. histolytica* strain K-9, from cultures with the help of time lapse cinemicrophotography. In their experimental conditions the nucleus of the amoeba could not be seen. Their description of the cytoplasmic division supports the observation of HARRIS[3] and adds further details to the process. Usually the trophozoites of *E. histolytica* divided into 2 equal halves, following a parallel procedure described above[3]. Mostly the division was completed within 3–8 min. Sometimes, 1 of the 2 sister amoebae divided into a 'normal' and a 'very small' sized amoeba (Figure 3, p. 170 of SHAFFER et al.[27]). The latter also behaved as a typical *E. histolytica*. Occasionally the 3 daughter amoebae, thus produced, were of the same size (see Figure 1, p. 568 of HARRIS[3]). SHAFFER et al.[27] have also frequently observed a process suggestive of conjugation of 2 *E. histolytica* trophozoites. 2 amoebae became apposed to each other and the membranes at their point of contact appeared to break; a few seconds after, the 2 amoebae separated. In the following, attempts have been made to follow the cytokinesis of *E. invadens* in cultures.

Materials and methods. Monoxenic cultures of *E. invadens* strain BC was grown in biphasic 'HSre + S' and 'HShsm + S' media at 24 °C. Further details of culturing and the history of the strain of amoeba used are given elsewhere[28]. During the log phase of growth[28], the amoebae were examined in paraffin sealed smears under a Wild M20 phase contrast microscope at room temperature (22 °C).

Results. Figure 1 is a photomicrograph of *E. invadens* BC taken from a 10-day-old culture. When the amoeba

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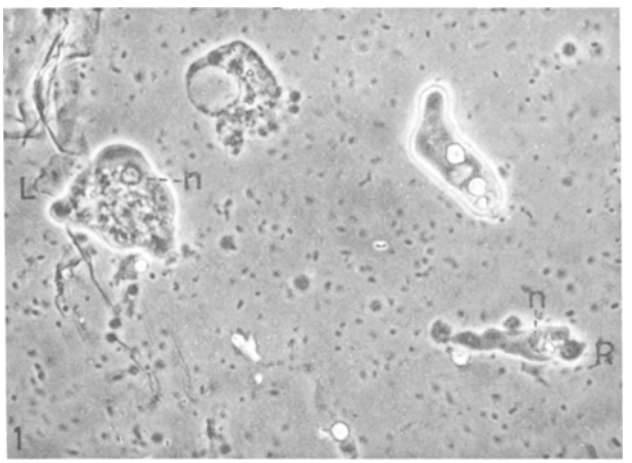
was first seen (5.39 p.m.), the 2 separating portions of the amoeba were unequal (Figure 1). The bigger portion on the left (L) which is about $^2/_3$ of the mother amoeba, contained one nucleus and many bacteria. On the right hand side (Figure 1, R), the smaller portion is about $^1/_3$ of the entire amoeba containing a nucleus too, and more prominent in Figure 2 (photomicrographed at 6.10 p.m.). The length of the attachment varied with the active movement of the 2 separating portions. Both of them produced pseudopodia, sometimes moving towards each other or tending to move apart. This 'to and fro' movement continued for more than $^1/_2$ h (41 min) after the first observation. During this period, the fine connection between the unequal portions was always found to be stretched and never relaxed. The points of contact of this fine cytoplasmic connection with the respective portions appeared shifting their positions with the movement of the 2 separating amoebae.

10 min after the second photomicrograph (Figure 2) was taken, while the 2 separating portions were approaching each other, the connection between them shortened and suddenly broke (6.20 p.m.). Just after the 2 amoebae were completely separated, they moved towards each other and seemed to fuse at the point of contact. After a few seconds the 2 amoebae separated. The bigger amoeba resulting from the foregoing division was actively moving and kept under observation for more than $^1/_2$ h. No further division could be detected in this recently divided amoeba. During the present investigation, unequal cytoplasmic division was usually observed in *E. invadens* BC, except on a few occasions. This type of unequal cytoplasmic division was also observed in *E. ranarum* and *E. moshkovskii* from similar cultures. A typical case is noted in the Figures 1 and 2.

Discussion. It is evident from the foregoing account that the nucleus of *E. invadens* divides well before the cytoplasm divides into 2. Both the nuclei of the dividing trophozoite, as seen under the phase microscope, are typical, with the central endosome (karyosome) and peripheral chromatin, thus strongly suggesting the interphase condition of the nuclei at the time of cytokinesis.

The unequal binary fission (cytoplasmic) of *E. invadens* is difficult to explain. Each of the sister amoebae is equipped with a nucleus of similar size. The various schemes of unequal cytoplasmic division put forward by MAZIA[2] (p. 316), do not appear to help us understand the state of affairs observed in *E. invadens* because the organellae, i.e. the typical mitotic apparatus, spindle, and asters etc., through which the mechanism of unequal cytoplasmic division may operate, are as yet not known in *Entamoeba*.

Active movement of the sister amoebae in opposite directions is an important factor in cytokinesis of amoeboid cells[2,29,30]. It has been observed that the fine connection between the 2 sister amoebae of *E. invadens* breaks when they are actually moving towards each other. Therefore, it appears that the movement in opposite



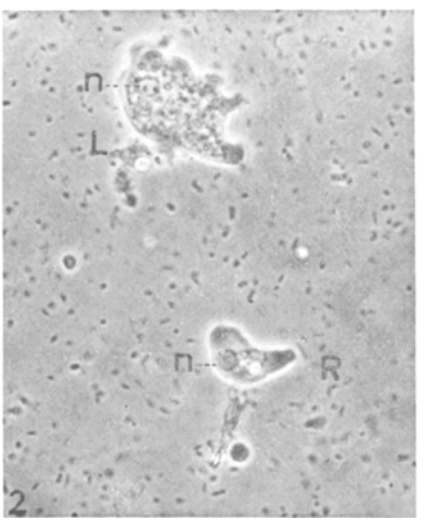


Fig. 1 and 2. *E. invadens* BC clone from a 10-day-old culture. 2 unequal portions (L and R) of the dividing amoeba, each containing a nucleus (n). Photomicrographed at an interval of 31 min. × 700. For further explanation see text.

Nuclear division in the genus *Entamoeba*[a]

Species	Trophozoite	Cyst	References
1. *E. histolytica*	+	+	5, 6
	+		4, 7–10
		+	11
2. *E. coli*	+		12
		+	13–16
3. *E. gingivalis*	+		17–20
	+	?+	21
4. *E. muris*	+	+	22
	+		23
		+	24
5. *E. invadens*	+		25
6. *E. ranarum*		+	26
7. *E. aulastomi*	+	+	31

[a] +, indicates the form studied; ?, indicates doubtfulness in the presence of a cyst in *E. gingivalis*.

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directions may not always be the immediate factor of separation.

Significance of apparent and temporary fusion of the 2 sister amoebae of *E. invadens* found in the present investigation and in *E. histolytica* by others[3,27] remains far from clear[31].

It should be stressed that all the observations noted above and recorded are from the in vitro cultures of *Entamoeba*. It remains to be ascertained whether they behave similarly in vivo[32].

Zusammenfassung. Bei *Entamoeba invadens* wurde eine ungleiche zellplasmatische Teilung beobachtet. Ein entsprechender Teilungsmodus gilt auch für *E. ranarum* und *E. moshkovskii*.

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[32] Acknowledgment. I am grateful to Prof. J. F. DANIELLI for providing the facilities and for his interest and encouragement. It is my pleasure to thank Dr. R. A. NEAL for the gift of initial cultures of amoebae and for his advice and help. Financial assistance of the Calcutta University and the Wellcome Foundation, London, is gratefully acknowledged.

Weitere Untersuchungen über den Parasitismus von Labyrinthula gegenüber Pilzen[1]

Morphologie und Lebensweise der Labyrinthulen sind so ungewöhnlich, dass diese Organismen keiner der bestehenden Klassen des Tier- oder Pflanzenreichs zugeordnet werden konnten. Eigene Untersuchungen ergaben, dass die labyrinthartig verzweigten Fadenplasmodien der Labyrinthulen aus Limax-Amöben entstehen. Kriterien, die von vegetativen Amöben abweichen, haben sich im Zusammenhang mit der Zystendifferenzierung dieser Amöben herausgebildet[2]. Die vegetativen Limax-Amöben leben stets einzeln. Erst nach der Differenzierung einer doppelten Hülle fusionieren die äusseren Plasmabezirke benachbarter Zellen. Dagegen grenzt sich das kernhaltige Zentralplasma jeder einzelnen Amöbe durch die Differenzierung der inneren Hülle ab und bleibt selbständig. STEY[3] wies ein Verbindungsorganell zwischen Zentralplasma und äusserer Hüllschicht nach.

Der Prozess der Zystendifferenzierung bedingt funktionelle Abwandlungen. Im Gegensatz zu den solitären Amöben, aus denen die Verbände der Labyrinthulen entstehen, nehmen sie keine geformte Nahrung auf und bewegen sich nicht amöboid. Ein sehr wesentliches Phänomen ist die parasitische Angriffskraft, die ihren solitären Amöben fehlt. Unlängst stellten wir fest, dass die Labyrinthulen in zahlreiche parasitische Pilze eindringen und deren Protoplasten zerstören können[4]. Wir untersuchten daher auch Vertreter dieser Organismengruppe aus anderen Gebieten.

Material und Methode. Im Oktober 1968 wurde Pflanzenmaterial (*Caulerpa sertularioides, Caulerpa cupressoides, Penicillus* sp., *Dictyota dichotoma, Syringodium* sp., *Thalassis* sp., *Cladophora* sp., *Acetabularia* sp.) in der atlantischen Litoralzone nahe Havanna (Arroyo Bermejo, Reñcon de Guanabo, Instituto de Oceanologia Habana, Playa de Baracoa) gesammelt (Wassertemperatur 27,5–29 °C). Die Methoden der Isolierung, Anreicherung und Kultivierung von Labyrinthulen aus Pflanzen wurden bereits beschrieben[5]. Als Agarboden wurde Meerwasseragar (Salzgehalt 3,5% und 1%) verwandt. Bevor die Labyrinthulen auf nährstoffreiche Agarböden übertragen werden, müssen sie frei von lebenden Bakterien sein. Dies wird durch Wanderung über flächig ausgebreitete, hitzegetötete Bakterien auf nährstoffarmen Meerwasser-Agarplatten erreicht.

Die parasitische Angriffskraft der Labyrinthulen wurde gegenüber *Curvularia* sp., *Fusarium vasinfectum, Fusarium oxysporum* var. *cubensis, Fusarium moniliforme, Colletotrichum gloeosporioides, Rhizoctonia solani, Pestalozzia* sp. und den Hauptpilzen *Madurella grisea, Histoplasma capsulatum* und *Trichophyton gallinae* untersucht. Die Hautpilze wurden bereits 5 Tage vor den Labyrinthulen auf die Kulturplatten übertragen.

Ergebnisse. Labyrinthula konnte nur von Cladophora-Algen aus Arroyo Bermejo, Playa de Baracoa und der Nähe des Ozeanologischen Instituts Havanna isoliert werden. Die Fadenplasmodien entwickelten sich auf Meerwasser-Agarplatten mit einem Salzgehalt von 3,5%. Aber auch Agarböden mit verdünntem Meerwasser (Salzgehalt 1%) waren gleichermassen geeignet. Für die Versuche mit Pilzen diente Meerwasseragar mit 1% Salzgehalt (pH 5,7), auf dessen Oberfläche hitzegetötete Bakterien flächig ausgespatelt waren. Das Pilzwachstum nahm seinen Ausgang von einem Nährboden (Biomalz- oder Sabouraud-Agar), der in ein kleines Fenster (etwa 1×2 cm) nahe der Plattenperipherie gegossen wurde. Die Labyrinthulen begannen ihre Wanderung im gegenüberliegenden Bereich der Platte. Sie sammelten sich an den Hyphen der Pilze und drangen in den meisten Fällen in sie ein. In den Hyphen zeigten sie starkes Wachstum und befielen schnell alle Verzweigungen des Myzels. Mit Hilfe ihrer Enzyme zerstörten sie die Protoplasten in den Hyphen, aber nicht die Zellwände. Die Myzelien solcher Kulturen erscheinen transparent und können leicht von den dicht bewachsenen Kontrollplatten unterschieden werden.

Die in Kuba isolierten Labyrinthulen zeigen grösste parasitische Aktivität gegenüber *Fusarium oxysporum* var. *cubensis, Fusarium moniliforme, Fusarium vasinfectum, Pestalozzia sp.* und *Colletotrichum gloeosporioides*. Intensiv geschädigt werden auch *Rhizoctonia solani* und der Hautpilz *Trichophyton gallinae*. Nur *Curvularia* sp., *Madurella grisea* und *Histoplasma capsulatum* werden von diesen Labyrinthula-Stämmen nicht zerstört.

Diskussion. Die erstmalig in Kuba beobachteten und kultivierten Labyrinthulen entfalten gegenüber wichtigen

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