

CLASSIFICATION OF AMOEBAE BELONGING TO THE ORDER AMOEBIDA WITH SPECIAL REFERENCE TO PATHOGENIC FREE-LIVING FORMS

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A SYSTEM of classification of the order Amoebida Kent, 1880, based on stable characters and probable phylogenetic relationships has yet to be developed.

The classification on general shape of an amoeba and superficial characters of locomotion are dangerous to use because environmental factors of various kinds exert profound changes in the form of an amoeba. Thus Dobell¹ states (p. 171)—“*A. fluvialis* is an amoeba of medium size. Its general appearance is somewhat like that of a small *proteus* amoeba, though ectoplasm is more strongly developed. The pseudopodia at times are large and flattened, and unlike those of typical *proteus* forms. At times also the organisms resemble *limax* amoebae, with a single large anterior pseudopodium and slug-like creeping movements. I have also seen forms which possessed pseudopodia like those of *A. vespertilio*. In fact, *A. fluvialis* is so variable—especially as regards its pseudopodia—that it is impossible to select as ‘typical’ any one of its many shapes, pseudopodia, or movements.” Minchin² says (p. 217)—“Not only may the characters of pseudopodia vary in different phases of the life-cycle, as already stated in the case of *Amoeba proteus*, but in the same phase under the influence of different media. Thus, no two forms of amoeba could appear more distinct at first sight than the *limax* and *radiosa* forms, originally regarded as distinct species. In the *limax*-form the whole body flows forward as a single pseudopodium, gliding along like a slug; in the *radiosa*-form the spherical body becomes star-like, sending out sharp-pointed pseudopodia on all sides. Nevertheless Verworn showed that one form could be changed into the other by differences in the medium. Doflein obtained similar form changes in *Amoeba vespertilio*, and showed that the body-form and characters of the pseudopodia were quite inadequate features for distinguishing the species of amoeba, depending as they do upon the conditions of the environment and nature of the medium.

Notwithstanding these comments, Schaeffer³ maintained that the change of form which

amoebae undergo is a fundamental morphological characteristic and forms the basis of natural classification. Bovee (see Bovee and Jahn⁴) has been attempting to develop Schaeffer's classification further and adding new genera using mainly pseudopodial types and trophic structure as the important criteria.

Kudo⁵, in a critical review of Schaeffer's classification, remarks that a genus must be founded on distinctive diagnostic characters by which it is distinguished from other genera without any difficulty by any competent worker. He says (p. 482)—“Time does not permit further examination; suffice it to say that these characterizations of the genera are so ambiguous, indistinct, and lacking sharp demarcation that they are impossible to follow in practice. This is my experience.”

Although Page (see Page⁶ for his earlier references) has been attempting to correlate pseudopodial, nuclear, cystic and flagellate formation characters in amoebae in the creation of genera, he has recognized *Hartmannella*, *Acanthamoeba*, *Flabellula*, *Vannella* and *Hyalodiscus* solely on pseudopodial characters and movement in amoebae. As the characters of pseudopodia and of locomotion are insufficiently stable, Wenyon⁷, Calkins⁸, Kudo⁹ and others have attempted to classify amoebae into families on the basis of such characters as the production of temporary flagella, their parasitic or free-living nature, and the presence of an accessory body (Nebenkörper; see Singh¹⁰ for the earlier literature). Although these systems are neither sound nor, probably, of any phylogenetic value, they are, unfortunately, still retained in most recent text-books of protozoology. Since some strains of free-living amoebae belonging to the genera *Hartmannella* and *Naegleria* are pathogenic to laboratory animals and man (see Singh and Das¹¹, Culbertson¹² and Chang¹³ for the literature), it has become very difficult to decide whether amoebae are parasitic or not.

A careful study of the literature dealing with nuclear division in amoebae clearly shows that conflicting views have been put forward by different workers who carried out cytological

investigations. The confusion that prevails can be assigned to two principal causes: first, difficulties in getting the stages of normal nuclear division, and secondly, the use of haematoxylin and non-specific aniline dyes to examine dividing nuclei. By the use of the culture method developed by Singh (see Singh¹⁰), it is easy to get all the normal stages of nuclear division in small free-living amoebae whenever desired without relying on chance. The discovery of the Feulgen reaction has made it possible to locate the chromosomal chromatin (DNA) in the resting nucleus of amoebae, and to trace its behaviour during nuclear division.

It is becoming increasingly evident that amoebae, whatever their size or nuclear structure, and whether they are uni or multi-nucleate and parasitic or not, can be placed in three main groups based on the mode of nuclear division. In the first group Feulgen-negative nucleolus or nucleoli give rise to polar masses and the nuclear membrane persists throughout division; in the second group Feulgen-negative granules or nucleoli do not give rise to polar masses and the nuclear membrane remains intact; in the third group Feulgen-negative nucleolus or nucleoli and the nuclear membrane disappear. In all the three groups the pre-existing Feulgen-positive chromatin gives rise to chromosomes, and at the end of nuclear division, the mass of fused chromosomes disperses and returns to the position seen in a resting nucleus. It may be emphasized that the nuclear division in amoebae is not of a primitive nature, nor there is much diversity of type. There is no justification for writers of text-books on biology to show an amoeba dividing by amitosis or in some such primitive way, as was thought about a hundred years ago.

These three distinct types of nuclear division provide a logical basis for dividing the order Amoebida into three families, as has been done by Singh¹⁰ and Singh and Das¹¹. Such a system of classification throws light on the probable trends of evolution of amoebae. Chang¹³ says that Singh's¹⁰ phylogenetic classification should be used as a basis in the taxonomic treatment of small, free-living amoebae. Culbertson¹² found mitotic characters as most helpful in classifying pathogenic soil amoebae and many other morphological features were rather inconsistent, depending upon differing conditions.

SCHIZOPYRENIDAE SINGH, 1952 EMEND, SINGH AND DAS, 1970

The resting nucleus contains a more or less central Feulgen-negative nucleolus or several Feulgen-negative nucleoli, which during mitosis form 'polar masses'. Nuclear membrane persists throughout division, 'Interzonal bodies' may be present. Amoebae may have more than one nucleus, and some genera may produce flagellate stage.

Type genus: *Schizopyrenus* Singh, 1952.

Feulgen-negative nucleolus dividing during mitosis to form 'polar masses'. Temporary flagella are not produced.

Type species: *Schizopyrenus russelli* Singh, 1952

The family Schizopyrenidae was named after the type genus *Schizopyrenus* in accordance with the International Rules of Zoological Nomenclature. *Schizopyrenus* was selected as the type genus because the amoebae included in it do not produce temporary flagella.

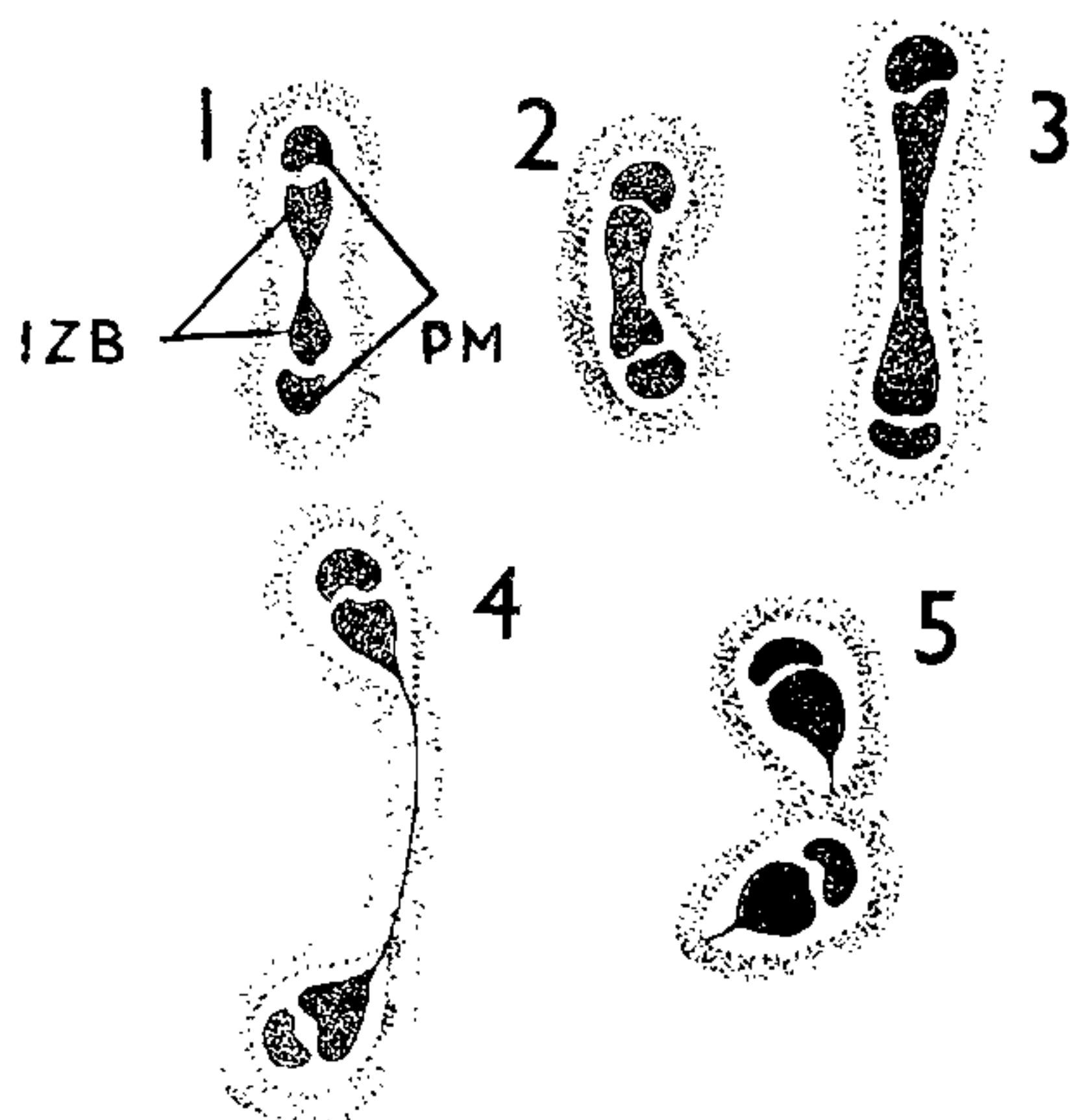
Other genera included in the family Schizopyrenidae: *Naegleria* Alexeieff emend. Singh, 1952; *Didascalus* Singh, 1952; *Tetramitus* Perty emend. Singh and Das, 1970; *Trimastigamoeba* Whitmore emend. Singh and Das, 1970; *Heteramoeba* Droop, 1962 emend. Singh and Das, 1970 and *Sappinia* (Dangeard).

Earlier Systems of Classifying Amoebae Included in the Family Schizopyrenidae

The creation of a family for amoebae that produce temporary flagellate stage is not justified. It seems more logical to put species such as *Didascalus thurtoni* Singh, 1952 and *Schizopyrenus russelli*, whose nuclear divisions are indistinguishable, in the same family rather than in different families merely on the ground that the former produces temporary flagella and the latter does not. The character of flagella production then becomes of generic value only. Singh¹⁰ used the presence or absence of interzonal bodies as a diagnostic character in recognizing the genera *Naegleria* and *Didascalus* Singh, 1952, both producing temporary flagella.

Vahlkampff¹⁴ was the first to discover the presence of polar masses during division in a limax amoeba. From his figs. 13 and 18 to 21, plate 6 (see Figs. 1, 2, 3, 4 and 5), it is certain that interzonal bodies were present, although no temporary flagella were recorded by him. Chatton and Lalung-Bonnaire¹⁵ created the genus *Vahlkampfia* for amoebae

possessing polar masses during nuclear division and pores in the cyst wall. From the nature of the nuclear division and the cyst wall we may conclude that Vahlkampff was dealing with *Naegleri gruberi* or some other *Naegleria* sp. with both amoeboid and flagellate forms.



FIGS. 1-5. Showing interzonal bodies (IZB) and polar masses (PM) during nuclear division in *A. limax* (from Vahlkampff¹⁴).

So far no amoeba with interzonal bodies but no temporary flagellate stage has been reported. Gläser¹⁶ in *N. tachypodia* figured distinct interzonal bodies but did not record the presence of temporary flagellate stage. When this amoeba was reinvestigated by Pietschmann¹⁷ (Fig. 6), she had no difficulty in finding the temporary flagella, although she wrongly placed the organism in the genus *Vahlkampfia*.

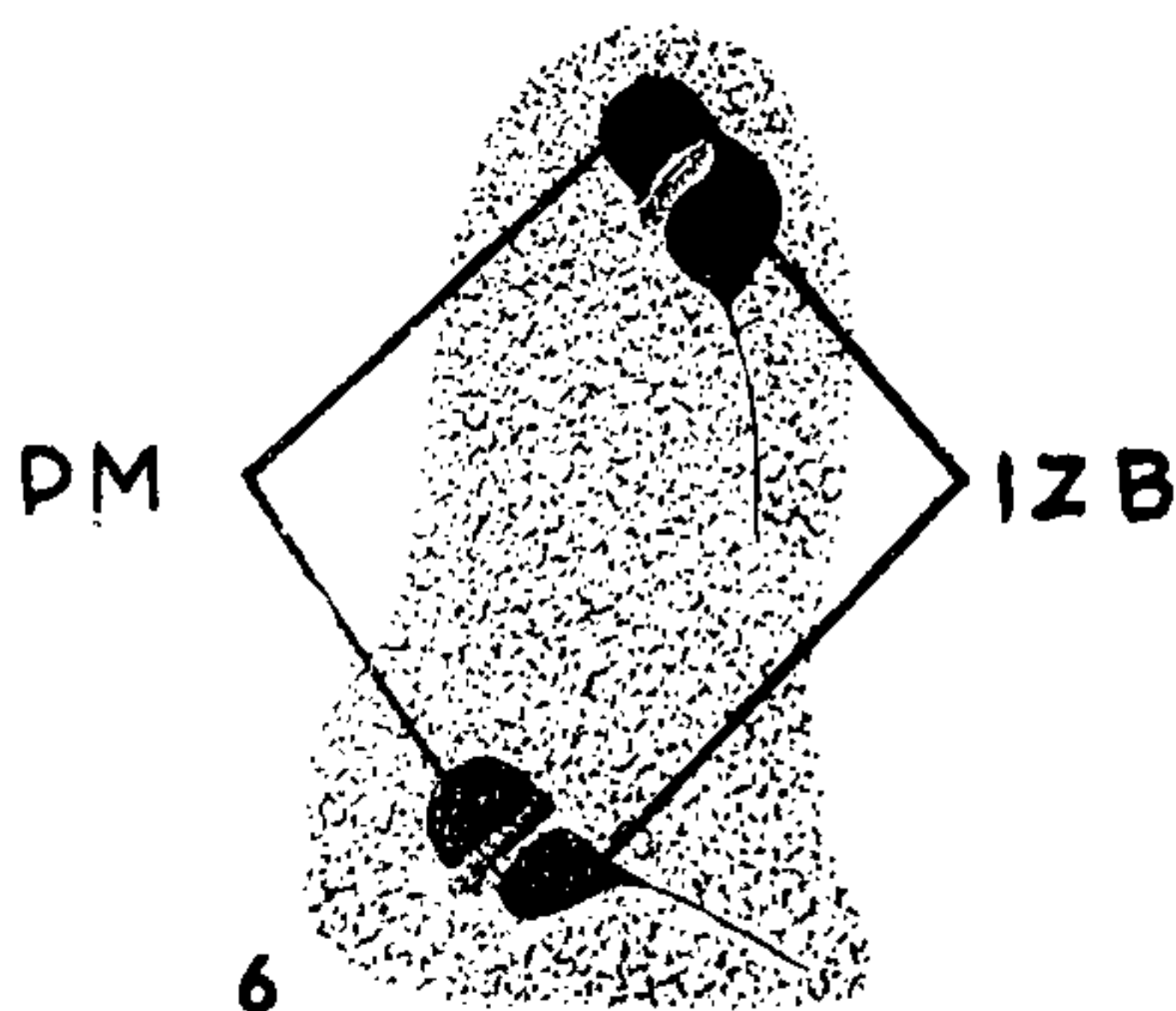


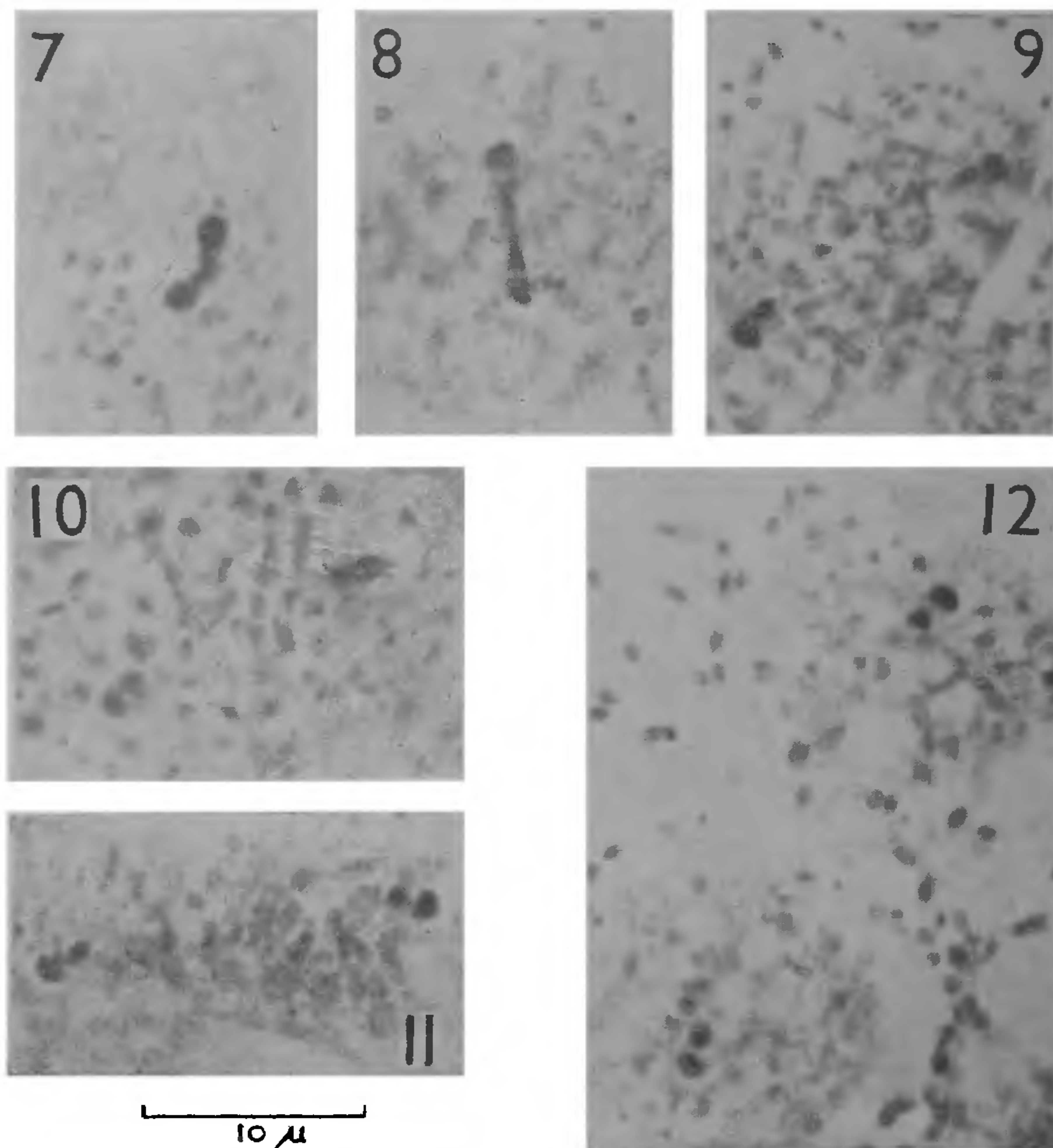
FIG. 6. Showing interzonal bodies (IZB) and polar masses (PM) during nuclear division in *N. tachypodia* from Pietschmann¹⁷).

Calkins¹⁸, unaware that Vahlkampff¹⁴ was dealing with *Naegleria* sp., suggested that amoebae possessing polar masses but no flagellate stage should be included in the genus *Vahlkampfia* and those with both polar masses and a temporary flagellate stage in the genus *Naegleria* Alexeieff. If the family Vahlkampfiidae, based on the type genus *Vahlkampfia* (Jollos 1917; Zulueta, 1917), is recognized, as has been done by Page¹⁹ and others, it will include only those amoebae that have a temporary flagellate stage; for these amoebae Kudo⁹ has erected the family Naegleridae. If an amoeba is found that possesses polar masses, interzonal bodies and no temporary flagellate stage, the genus *Vahlkampfia* will have to be recognized, and included, along with other genera in the family Schizopyrenidae.

Page¹⁹ takes the view that the occurrence of interzonal bodies is too uncertain and unimportant to justify generic separation, and suggests that the genus *Naegleria* should be defined as suggested by Calkins¹⁸.

Rafalko²⁰ was the first to give a convincing and good account of the Feulgen-negative interzonal body from the time it is first seen and its behaviour during the subsequent stages in nuclear division in *N. gruberi*. Singh¹⁰ confirmed the findings of Rafalko, using Feulgen reaction. Chang,¹³⁻²¹ Butt, Baro and Knorr²², Culbertson, Ensminger and Overton²³, Culbertson¹² and Singh and Das¹¹ all found that interzonal bodies were consistently present in dividing *Naegleria* and have placed this genus in the family Schizopyrenidae. As interzonal bodies are constantly present in both *N. gruberi* and *N. aerobia* Singh and Das, 1970 (Figs. 7, 8, 9, 10, 11 and 12), an amoeboid-flagellate isolated from a fatal human case of primary amoebic meningo-encephalitis, it seems reasonable to separate the genera *Naegleria* and *Didasculus*, both of which have temporary flagella, on the basis of presence or absence of interzonal bodies. Carter²⁴ has isolated an amoeboid-flagellate from two human cases of amoebic meningo-encephalitis in Australia and has named it *N. fowleri* sp. nov. This amoeba appears similar to *N. aerobia* in cultural requirements and cyst morphology. If *N. fowleri* has no interzonal bodies, as claimed by Carter, this amoeba should be placed in the genus *Didasculus* in the family Schizopyrenidae.

N. aerobia has been isolated from five different sewage sludge samples in Lucknow by Singh



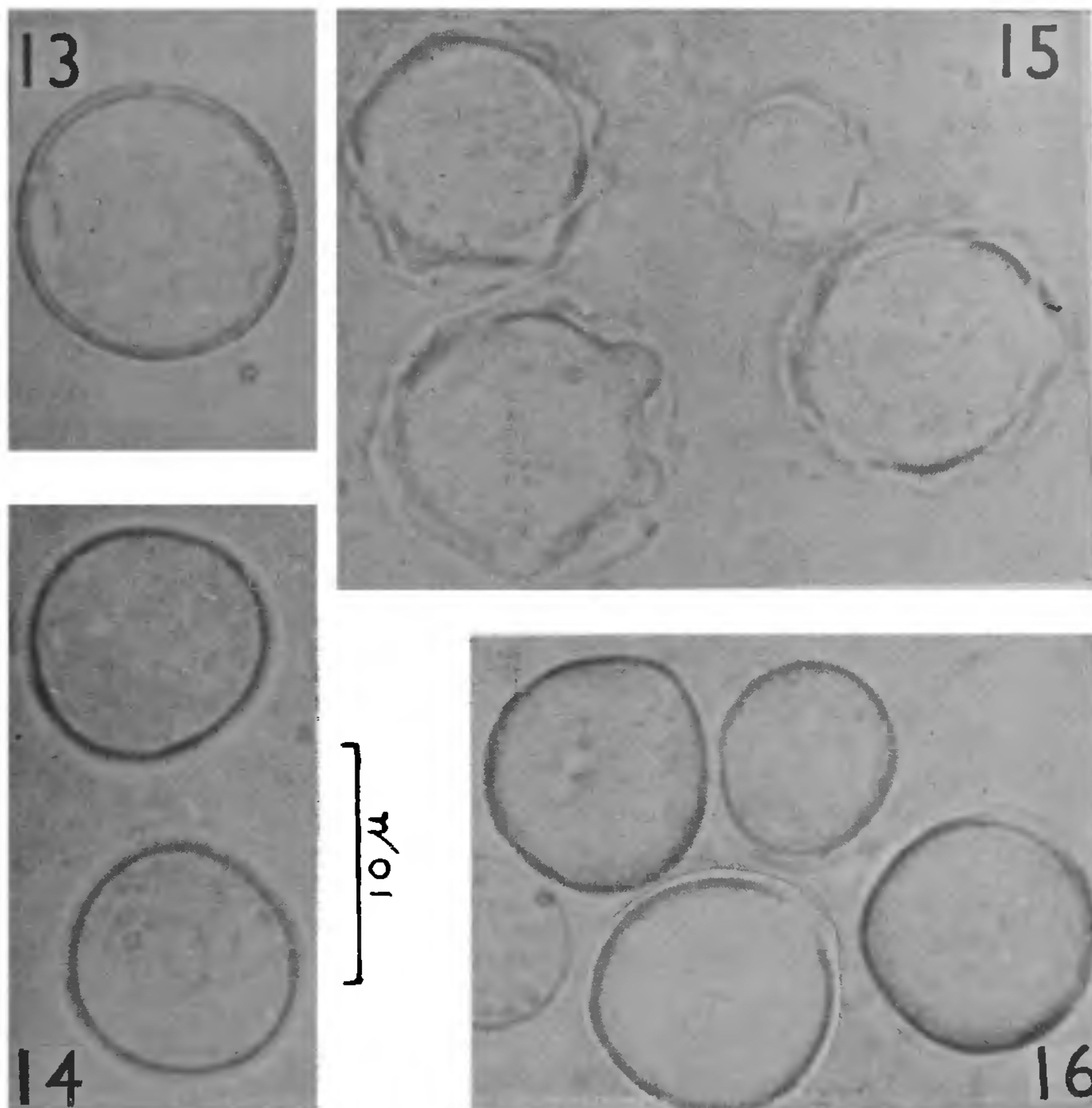
FIGS. 7-12. Showing the appearance of interzonal body and its behaviour during subsequent stages in nuclear division in *N. aerobia* (from Singh and Das¹¹).

and Das²⁵. It is pathogenic to mice when given intranasally. This is the first record of *N. aerobia* in a free-living stage and is of considerable epidemiological importance. A number of strains of *N. gruberi* isolated from soil and fresh water were found to be non-pathogenic to mice (see also Singh and Das¹¹) (Figs. 13 and 14).

Chang¹³ has recognized the family Schizopyrenidae and has included the genera *Schizopyrenus*, *Didascalus* and *Naegleria* in this family.

ENDAMOEBIDAE CALKINS EMEND. SINGH AND DAS, 1970

The resting nucleus contains a Feulgen-positive karyosome or Feulgen-positive chromatin granules. Feulgen-negative granules or nucleoli do not give rise to 'polar masses', and the nuclear membrane is always intact during mitosis. Amoebae may be uni- or multinucleate, and no temporary flagellate stage has been discovered.



FIGS. 13-16. Fig. 13. Living cyst of *N. gruberi* showing double cyst wall with pores. Fig. 14. Living cysts of *N. aerobia* with a single wall whose outside consists of a fairly thick transparent gelatinous layer. No pores could be seen. Fig. 15. Living cyst of *H. rhysodes* with two walls. The outer wall has folds and ripples and is loosely applied to the inner wall, which is irregularly stellate, with truncated rays, or irregularly polyhedral in appearance. Pores plugged with a structureless substance can be seen. Fig. 16. Living cysts of *H. cuibertsoni* with two walls perforated by pore plugged by a structureless substance. The outer wall is nearly circular or irregular in outline.

Type genus: *Entamoeba* Casagrandi and Barbagallo emend. Singh and Das, 1970

The resting nucleus has a relatively small Feulgen-positive karyosome located in or near the centre and Feulgen-negative peripheral granules lining the nuclear membrane. During mitosis the peripheral granules do not give rise to 'polar masses' and the nuclear membrane is always intact. No temporary flagella are produced.

Type species: *Entamoeba histolytica* (Schaudinn)

Genus *Endamoeba* Leidy emend. Singh and Das, 1970

The resting nucleus has Feulgen-positive chromatin without a karyosome and Feulgen-negative nucleoli.

The genera *Hydramoeba* Reynolds and Looper, *Iodamoeba* Dobell and other genera,

in which polar masses are not produced and the nuclear membrane persists throughout division, can be included in the family Endamoebidae.

There is a great confusion in the study of nuclear division in amoebae placed in the family Endamoebidae. The culture method of growing *Entamoeba histolytica* and other anaerobic amoebae for the study of their nuclear division, developed by Dubey and Das²⁶, will be helpful in determining the normal stages of nuclear division in these amoebae. The review of nuclear division in *Entamoeba* by Neal²⁷ clearly shows that different stages of mitosis are not clearly understood.

The work of Noble²⁸ on *E. gingivalis*, Wenrich²⁹ on *E. muris*, Narasimhamurti³⁰ on *E. invadens*, Pan³¹ on *Iodamoeba bütschlii*, belonging to Endamoebidae, has shown that the Feulgen-positive chromatin gives rise to chromosomes and the nuclear membrane persists throughout division. The behaviour of Feulgen-negative granules or nucleoli during mitosis is not clearly understood. It is possible that they may help in the formation of spindle.

HARTMANNELLIDAE VOLKONSKY, 1931

EMEND. SINGH, 1952

Volkonsky³² created a subfamily Hartmannellinae in the family Amoebidae to include only such amoebae whose resting nucleus contains a single nucleolus. Singh¹⁰ raised this subfamily to the status of a family, considering that the form of nuclear division justified it.

The family Hartmannellidae was created on the well-known genus *Hartmannella*.

The resting nucleus has either a single Feulgen-negative nucleolus or several nucleoli. During mitosis the nucleolus, or nucleoli, disappear, and a spindle with chromosomes arranged as an equatorial plate resembling those found in higher animals and plants develops. The nuclear membrane disappears during mitosis. Amoebae may be uni or multinucleate; no temporary flagella have been discovered.

Type genus: *Hartmannella* Alexeieff. emend. Singh, 1952

The resting nucleus contains a single Feulgen-negative nucleolus. During mitosis the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed. No temporary flagella are produced.

Type species: *Hartmannella glebae* (Dobell)
Genus *Amoeba* Ehrenberg emend. Singh and Das, 1970

Amoebae uninucleate. Resting nucleus contains several Feulgen-negative nucleoli. During mitosis the nucleoli disappear and a spindle with chromosomes arranged as an equatorial plate is formed.

Type species: *Amoeba proteus* (Pallas)

The mitotic division in *A. proteus* has been described by Liesche³³.

Genus *Pelomyxa* Greeff emend. Singh and Das, 1970

Amoebae multinucleate; division by plasmotomy. Resting nucleus contains several Feulgen-negative nucleoli. During mitosis the nucleoli disappear and a spindle with chromosomes arranged as an equatorial plate is formed.

Type species: *Pelomyxa carolinensis* Wilson, 1900

Kudo³⁴ has given a good account of nuclear division in *P. carolinensis*.

Genus *Dobellina* Bishop and Tate. emend. Singh and Das, 1970

Amoebae multinucleate; division by plasmotomy. The resting nucleus contains a single Feulgen-negative nucleolus. During mitosis the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed.

Type species: *Dobellina mesnili* (Keilin)

An account of the nuclear division in *D. mesnili* has been given by Bishop and Tate³⁵.

Other genera of amoebae in which the Feulgen-negative nucleolus or nucleoli and nuclear membrane disappear during mitosis and a spindle with chromosomes arranged as an equatorial plate is formed may be placed in Hartmannellidae.

Remarks on the Inclusion of Genera in the Family Hartmannellidae

Volkonsky³² placed three genera in the subfamily Hartmannellinae; *Hartmannella* (type *H. glebae* Dobell, 1914) in which the spindle is barrel-shaped or cylindrical and the cyst wall is smooth or lightly folded; *Glaeseria* (type *G. testudinis* Ivanic, 1926), with the same spindle shape, but with division occurring in the cyst; and *Acanthamoeba* (type *A. castellanii* Douglas, 1930) for amoebae with

conical, pointed-ended spindle and rough cyst wall.

Singh¹⁰ suggested that spindle shape was an inadequate character for separating the genera *Hartmannella* and *Acanthamoeba*, and established the genera *Hartmannella* for amoebae having a single Feulgen-negative nucleolus and showing true mitosis. Singh and Das¹¹ have found both globular and somewhat pointed spindles in *H. castellanii*. Ray and Hayes³⁶ and Adam³⁷ have agreed with Singh.

Page^{19,38,39}, however, considers that the separation of *Hartmannella* and *Acanthamoeba* is both necessary and justified. He¹⁹ has defined the genus *Hartmannella* as follows (p. 519): "Amoebae in active locomotion with one or, briefly, more broadly digitiform or hemispherical lobose pseudopods, sometimes with small filamentous projections but never with well-developed conical or tapering pseudopods; most active locomotive form *limax*-like. Locomotion not strongly eruptive. Cyst may or may not be formed; where known, smooth-walled and rounded."

Page¹⁹ has set up *H. hyalina* Dangeard, 1900, as the type species of the genus *Hartmannella*, in spite of the fact that Dangeard's⁴⁰ description, based entirely on fixed preparations, does not refer to locomotion.

Sandon⁴¹, who found *H. hyalina* widespread in soils, describes it as follows (p. 133): "Active form very like *N. gruberi*, but usually slightly larger; protoplasm rather more fluid and pseudopodia generally formed 'eruptively', i.e., they burst suddenly through the surface of the amoeba and the granular protoplasm flows rapidly into the newly formed process; generally, instead of at once forming a long finger-like pseudopodium the eruption flows round the outside of the original surface of the body which ultimately becomes absorbed. The cysts are spherical; outer wall usually more or less wrinkled; walls not perforated; protoplasmic contents rounded lying quite freely within the wall." Reliance to create a genera based on imperfectly studied amoeba, as has been done by Page¹⁹, is of little value.

Page³⁸ has defined the genus *Acanthamoeba* as follows (pp. 721-22): "Amoebae in active locomotion with broad, anterior hyaline lobopodium from which are produced singly or 2's or 3's several or many slender, hyaline projections (acanthopodia) which taper to a finely rounded end; acanthopodia may move occasionally and eventually are resorbed by

advancing amoeba or remain on the surface until resorbed at posterior end. Locomotion by flow into anterior hyaline region, not markedly eruptive, slow. Cyst wall consists of endocyst, which is more or less polyhedral or stellate, and ectocyst, which may be wrinkled and more or less closely appressed to endocyst or circular in outline and showing only slight ripples. General form of the cyst thickly biconvex or polyhedral, depending on distribution of corners of endocyst; appearance generally more or less mammillated. Excystment by removal of operculum into interior of cyst from point of contact between endocyst and ectocyst, followed by exit of amoeba through opening at that point." Page³⁹ (p. 25) says: "Within the genus *Acanthamoeba*, the cysts, the very distinctive as generic characteristics, are somewhat more confusing as a means of distinguishing species." Page³⁸ has made *A. castellanii* the type species.

Page^{19,38,39} has separated *Hartmannella* from *Acanthamoeba* on the basis of pseudopodial and cyst characters. It may be of interest to point out that *H. glebae* does produce acanthopodia, but the cysts are rounded or spherical with smooth wall and without pores or opercula. The mode of excystment of this amoeba is quite different from that of *A. castellanii* (see Singh and Das¹¹). Sandon⁴¹ recorded *H. glebae* from several soils; he says (pp. 139, 140) that the pseudopodia are pointed, with ectoplasm often drawn out into thin web-like sheets between them; the cysts are spherical, with thick smooth outer wall without pores. Singh¹⁰ isolated *H. glebae* from Rothamsted soil and Chang⁴² from river and well water. On the basis of cyst character and mode of excystment, *H. glebae* cannot be included in the genus *Acanthamoeba*. Only on pseudopodial characters, can *H. glebae* be placed in the genus *Acanthamoeba*. The unreliability of the pseudopodial characters to distinguish *Hartmannella* from *Acanthamoeba* can easily be judged by the following remarks of Ray and Hayes³⁶ in the case of *H. astronyxis* (*A. astronyxis* according to Page³⁸). They say (p. 162): "Lobopods, filopods and micropseudopodia are all formed and may be present in the same individual at the same time. The kind of pseudopod produced is conditioned by many factors in the environment; osmotic pressure, oxygen tension, and contact with a solid substratum and with food particles are among the more important modifying conditions. Broad,

flat lobopods are commonly present during active locomotion in individuals which are in a liquid environment and in contact with a solid, smooth surface. In locomotion on agar surface, pseudopods tend to be narrow and tapering and may become extremely long and attenuated. In individuals suspended in a surface film or floating free, relatively short, narrow, radiating filopods are the more common pseudopodial types; these are often slowly bent and waved about. Under conditions of slightly lowered oxygen tension the *limax* shape is normally assumed. If the total salt concentration exceeds 0.5%, branched pseudopods are produced. Locomotion, and hence to a large degree the type of pseudopodia formed, depends also upon the nutritional state of culture and the stage of the life-cycle." Dobell¹ has also mentioned similar form changes in *A. glebae*. It seems that the creation of both the genera *Acanthamoeba* and *Hartmannella*, as done by Page^{19-38,39}, is very misleading and is not justified. *Acanthamoeba* is a synonym of *Hartmannella*. Any one recognizing the genus *Acanthamoeba* will have to recognize *H. glebae* as the type species in accordance with the International Rules of Zoological Nomenclature.

At this stage, it is more important to create species in the genus *Hartmannella*, based on sound characters, which can be identified with ease by any competent worker, than to split the genus *Hartmannella* into a number of genera. As suggested by Volkonsky³² the amoebae, like *A. mira* (Gläser, 1912) and *H. testudinis* (Ivanic, 1926), where nuclear division takes place in the cyst, may be taken out of the genus *Hartmannella* and placed in the genus *Glaeseria*. The mode of nuclear division places the genus *Glaeseria* in the family Hartmannellidae.

Chang¹³ has recognized the genera *Hartmannella*, *Acanthamoeba* and *Singhella*, a new genus, in the family Hartmannellidae. *Singhella* has been defined as follows: double-walled cysts; ecto- and endoplasm indistinguishable; many vacuoles (type *S. leptocnemus*). The genus *Singhella* has been created on the basis of *H. leptocnemus* Singh, 1952.

Among the hartmannellid amoebae, only *H. rhysodes* Singh, 1952 and *H. culbertsoni* Singh and Das, 1970 have been found so far to be pathogenic to laboratory animals (see Singh and Das¹¹). They are very widely distributed in soils, sewage sludge and other sub-

strates (see Singh and Das^{11,25} and Culbertson¹²) and can easily be distinguished by the characters of their cysts (Singh and Das¹¹) (Figs. 15 and 16). The validity of these two species has been recognized by Culbertson¹². They may be important in causing meningo-encephalitis in farm animals.

Nuclear Division and Phylogeny

Classification of amoebae on form, locomotion and pseudopodial characters and on parasitic or free-living nature is neither of probable phylogenetic value nor is based on stable characters. The classification of amoebae into families based on nuclear division throws light on the possible course of evolution in amoebae. In Schizopyrenidae the Feulgen-negative nucleolus or nucleoli give rise to polar masses during mitosis. The nuclear membrane persists throughout division. A form such as *N. gruberi*, which during division has polar masses and interzonal bodies and can readily produce temporary flagellate stage, may be regarded as a primitive amoeba. During the course of evolution it seems that amoebae lose the power to produce interzonal bodies, although they are able to produce temporary flagella, as is the case in *D. thorntoni*. In a form like *S. russelli* no temporary flagellate stage is produced, although the nuclear division of this amoeba is indistinguishable from *D. thorntoni*. Thus the primitive character of temporary flagella production in *S. russelli* is lost, but it retains the family character in having polar masses. In Entamoebidae Feulgen-negative granules or nucleoli do not give rise to polar masses and the nuclear membrane is intact during division. In Hartmannellidae the Feulgen-negative nucleolus or nucleoli and the nuclear membrane disappear during nuclear division, as happens in *H. glebae*, *D. mesnili*, *A. proteus* and *P. carolinensis*. A spindle with chromosomes arranged as an equatorial plate is formed, as in higher animals and plants. Judging from the advanced type of nuclear division in the family Hartmannellidae and the loss of the flagellate stage, it is tempting to put forward the theory that amoebae are evolved from flagellate ancestors and not the flagellates from amoebae.

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REGIONAL GRAVITY STUDIES OF THE DECCAN TRAP AREAS OF PENINSULAR INDIA *

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EXTENSIVE regional gravity surveys over the Deccan trap areas of Maharashtra and parts of Mysore, Andhra Pradesh and Gujarat in Peninsular India were conducted by the Geological Survey of India during the period, 1964-70, as part of its programme of investigations connected with the International Upper Mantle Project. An area of roughly 3,50,000 square kilometers bounded roughly by latitudes 15° N and 22° N and longitudes 72° 30' E and 79° E was covered by gravity observations with a station distribution of roughly one per 150 square kilometers. The objectives of these studies were (a) the estimation of the thick-

ness of traps and crustal thickness (b) the identification and investigation of major subsurface structural features depth that brought the magma to the surface and (c) the study of the degree of isostatic equilibrium and compensation in these areas. The results of seismic refraction soundings to estimate the thickness of traps at a number of locations over this trap territory together with the results of some east-west regional gravity profiles and detailed gravity-cum-magnetic investigations in the Koyna earthquake affected area were published in Volume 100 of the *Memoirs of the Geological survey of India* (Kailasam et al., 1969).

The regional geological features of the area are shown in the sketch map (Fig. 1) which is based on the geological map of India pub-

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