

***Igor B. Raikov***

# **The Protozoan Nucleus**

***Morphology and Evolution***

***Translated from the Russian***

***by Nicholas Bobrov***

***and Marina Verkhovtseva***

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## Preface

This book continues, somewhat, the author's monograph *Karyology of Protozoa*, which was published in Russian in 1967. Investigations of the nuclear apparatus of protozoans developed very rapidly in the 1960s and 1970s, when virtually the whole morphology of protozoan nuclei was transferred to the ultrastructural level. Our knowledge of protozoan mitosis also reached a newer, qualitatively higher level with electron microscopy playing the main role in this respect, also. A peculiar mechanism of mitosis in mesokaryotic protozoans (Dinoflagellida) was revealed, and extremely important discoveries were made in the field of ciliate macronuclei.

Hence, this book is not the second edition of *Karyology of Protozoa*, although it has the same plan, in part. Because an enormous amount of new material became available and many points had to be critically reviewed, the monograph was virtually rewritten for the 1978 Russian edition. When the text of this book was being prepared for the English translation, it was supplemented and worked over in accordance with the data obtained during 1977-1981. New illustrations were added, and some of the old ones were removed.

The author hopes this book will make it possible to use protozoan nuclei on a wider scale and give a better understanding of the karyology of lower eukaryotes within the framework of cell biology.

Leningrad, August 1982

I. B. RAIKOV

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## Introduction

KARL BĚLAŘ, an eminent protozoologist and cytologist, wrote the book *Der Formwechsel der Protistenkerne*, which was published in 1926. In it, he summed up the results of early research on the nuclear apparatus of protozoans and criticized the theories of the "dualism of the nuclear substance" and "chromidia", which were widely accepted at the time and which upheld the existence of anucleate (chromidial) stages in the life cycle of many protozoans. The publication of BĚLAŘ's book became a turning point in the development of the karyology of protozoans, since he was the first to consider and critically assess an enormous amount of very diverse literary material from a strictly scientific standpoint of general karyology and cytogenetics. BĚLAŘ drew the extremely important conclusion that the general laws of the replication and segregation of chromosomes can be fully applied to protozoans. Although the nondividing nuclei and the types of mitosis of protozoans seem to be very diverse, their chromosomes are characterized by a constant number, individuality, and regular segregation in mitosis.

The study of protozoan nuclei has greatly advanced since then. During the era of light microscopy, a thorough investigation was made of mitosis, meiosis, and the cycles of spiralization of chromosomes in some flagellates. The discoveries of that period included the alternation of the haploid and diploid generations in the life cycle of foraminiferans and the phenomenon of polyploidy in protozoans. The data obtained were included in textbooks and manuals of protozoology (DOFLEIN and REICHENOW 1953, GRELL 1956a, DOGIEL 1965, etc.) and in RAIKOV's book (1967a), which specifically dealt with the karyology of protozoans.

The methods of studying protozoan nuclei have substantially changed in the past 15 years. Prior to 1965-1967, the nuclear structure was investigated mainly by photomicroscopy, although some data were obtained by methods involving electron microscopy, autoradiography, and biochemistry. In the late 1960s and the 1970s, however, these methods underwent the greatest development. At present, all types of protozoan nuclei have an ultrastructural characterization. Instead of the old classification of the types of mitosis, a new one based on electron microscopy is now available. The fine structural cytochemistry of protozoan nuclei has also been markedly developed. The autoradiographic recording of the synthesis of certain substances in protozoan nuclei has been largely transferred to the ultrastructural level. Moreover, the biochemical studies of protozoan nuclei have advanced very rapidly. Some forms of Protozoa, especially the ciliate *Tetrahymena pyriformis*, are now frequently used by biochemists as a model of the eukaryotic cell in general. This field is now very large. Unfortunately, space does

not allow this author to thoroughly discuss some biochemical aspects of protozoan karyology, such as the nucleotide composition of the DNA of protozoans, the composition and properties of the histones of protozoan nuclei, and the arrangement of unique and repetitive DNA sequences in their genomes. These aspects are considered, for instance, in the multivolume treatise *Biochemistry and Physiology of Protozoa* (Second edition, Academic Press, 1979). We will deal mainly with the morphological and morphofunctional aspects of protozoan karyology. Molecular biochemical data will be given only when they help to explain the structural organization of the nucleus (e.g. the ciliate macronucleus).

No special monographs on protozoan karyology have been published recently. However, some structural and functional aspects of protozoan nuclei have been discussed in reviews of general protozoology (GRELL 1973, SLEIGH 1973). In this book, various aspects of protozoan karyology are considered to different extents. For instance, the behaviour of nuclei during sexual processes (copulation, conjugation, and autogamy) is hardly discussed. As for ciliates, the author wrote a special review (RAIKOV 1972d) concerning those processes. Among the phenomena concerned, only meiosis is considered in this book. Ample space is given to nuclear dualism and the macronuclear structure of ciliates. In this respect, the author presents literary as well as his own data.

Protozoans are cells as well as organisms. Their evolution (as regards the origin of some taxa from others) is the subject of protozoology as a constituent of zoology. Besides the purely "zoological" evolution, however, protozoans as cells definitely evolved with respect to their cellular structures and organoids, and this is the subject of evolutionary cytology. For instance, it has now been firmly established that not all protozoans are on the same level of evolution as regards their cell nucleus. Most protozoans are typical *eukaryotic* organisms with a formed nucleus that is covered with a two-membrane envelope and contains typical chromosomes rich in DNA and histones. This nucleus divides by mitosis, forming an achromatic apparatus of microtubules. But in at least one group of protozoans (Dinoflagellida) chromosomes have a structure similar to that of the nucleoids of *prokaryotic* organisms (bacteria, cyanophyceans, actinomycetes): they do not have the histone component and lack nucleosomal subunits. But the Dinoflagellida do have a formed cell nucleus with a typical envelope; it divides by mitosis, although a very unusual one (membranes participate in the segregation of chromatids). Because of this unusual aspect, this level of the organization of the protozoan cell was called *mesokaryotic* (DODGE 1965). Thus, protozoans are either eukaryotic or mesokaryotic. Prokaryotic protozoans have not been discovered yet. These two groups of protozoans should be considered separately, beginning with the eukaryotic forms.

For the reader's convenience, the classification of protozoans used in this book is given below. It is the system proposed by the Committee on Systematics and Evolution of the Society of Protozoologists (see LEVINE *et al.* 1980). The system is presented here with some minor changes and in a simplified version (e.g. the enumeration of orders is omitted when it is not essential). The classes of myxomycetes (Acrasea, Eumycetozoea, and Plasmodiophorea), which cannot be regarded as true protozoans, are not discussed in this book. Widely used synonyms and trivial names of some taxa are given in parentheses.

## Subkingdom Protozoa

Phylum I. *Sarcomastigophora*Subphylum *Mastigophora* (Flagellata, flagellates)Class 1. *Phytomastigophorea* ("plant" flagellates)

Orders: *Chrysomonadida*, *Prymnesiida*, *Heterochlorida*, *Cryptomonadida*, *Dinoflagellida*, *Euglenida*, *Chloromonadida*, *Volvocida* (*Phytomonadida*), *Prasinomonadida*, *Silicoflagellida*

Class 2. *Zoomastigophorea* ("animal" flagellates)

Orders: *Choanoflagellida* (collar flagellates), *Proteromonadida*, *Bicosoecida*, *Retortamonadida*, *Rhizomastigida*, *Kinetoplastida*, *Diplomonadida*, *Oxymonadida*, *Trichomonadida*, *Hypermastigida*

Subphylum *Opalinata*Subphylum *Sarcodina*Superclass *Rhizopoda*Class 1. *Lobosea*

Subclass *Gymnamoebia* (naked amoebae)

Orders: *Schizopyrenida*, *Amoebida*, *Pelobiontida*

Subclass *Testacealobosia* (Testacea, testate amoebae)

Orders: *Arcellinida*, *Trichosida*

Class 2. *Acarpomyxea*

Orders: *Leptomyxida*, *Stereomyxida*

Class 3. *Acrasea*Class 4. *Eumycetozoea*Class 5. *Plasmodiophorea*Class 6. *Filosea*

Orders: *Aconchulida*, *Gromiida*

Class 7. *Granuloreticulosea*

Orders: *Athalamida*, *Monothalamida*, *Foraminiferida*

Class 8. *Xenophyophorea*Superclass *Actinopoda* (classes 1-3 are radiolarians)Class 1. *Acantharea*Class 2. *Polycystinea*

Orders: *Spumellarida*, *Nassellarida*

Class 3. *Phaeodarea*Class 4. *Heliozoa* (heliozoans)

Orders: *Desmothoracida*, *Actinophryida*, *Taxopodida* (*Sticholonchida*), *Centrohelida*

Phylum II. *Labyrinthomorpha* (labyrinthulids)Phylum III. *Apicomplexa*Class 1. *Perkinsea*Class 2. *Sporozoea* (sporozoans)

Subclass *Gregarinia* (gregarines)

Orders: *Archigregarinida*, *Eugregarinida*, *Neogregarinida*

Subclass *Coccidia*

Orders: *Agamococcidiida*, *Protococcidiida*, *Eucoccidiida* [with suborders *Adeleina*, *Eimeriina* (coccidians s. str., toxoplasms and sarcosporidians), *Haemosporina* (malaria parasites)]

Subclass *Piroplasmia* (piroplasmids)

Phylum IV. *Microspora* (microsporidians)Class 1. *Rudimicrosporea*

Order: *Metchnikovellida*

Class 2. *Microsporea*Orders: *Minisporida*, *Microsporida*Phylum V. *Ascetospora*Class 1. *Stellatospora* (Haplosporea, haplosporidians)Orders: *Occlusosporida*, *Balanosporida*Class 2. *Paramyxea*Phylum VI. *Myxozoa*Class 1. *Myxospora* (myxosporidians)Orders: *Bivalvulida*, *Multivalvulida*Class 2. *Actinospora* (actinomyxidians)Phylum VII. *Ciliophora* (ciliates, infusorians)Class 1. *Kinetofragminophorea*Subclass *Gymnostomatia*Orders: *Primociliatida*, *Karyorelictida*, *Prostomatida*, *Pleurostomatida*Subclass *Vestibulifera*Orders: *Trichostomatida*, *Colpodida*, *Bursariida*, *Platyophryida*, *Entodiniomorphida*Subclass *Hypostomatia*Orders: *Synhymeniida*, *Nassulida*, *Cyrtophorida*, *Chonotrichida*, *Rhynchodida*, *Apostomatida*Subclass *Suctoria*Class 2. *Oligohymenophorea*Subclass *Hymenostomatia*Orders: *Hymenostomatida*, *Scuticociliatida*, *Astomatida*Subclass *Peritrichia*Class 3. *Polyhymenophorea*Subclass *Spirotrichia*Orders: *Heterotrichida*, *Odontostomatida*, *Oligotrichida*, *Hypotrichida*

## 1. Morphology of Eukaryotic Protozoan Nuclei

All Protozoa have at least one nucleus. Descriptions of anucleate (chromidial) protozoans found in the old literature are now of purely historical interest. Protozoan nuclei consist of the same structural components as the nuclei of metazoan cells: the nuclear envelope, chromatin, nucleoli, karyolymph, and various intranuclear inclusions.

The nuclei of mesokaryotic protozoans (dinoflagellates) are not considered in this chapter because they will be dealt with in Chapter 4. Highly polyploid nuclei of radiolarians and the macronuclei of ciliates are also discussed in other chapters (Chaps. 6-8), but they will be dealt with in this chapter in terms of the structure of their nuclear envelopes.

### Number, Shape, and Size of Nuclei

Most Protozoa are uninucleate. Almost all Mastigophora (except some forms, mainly parasitic ones, of the orders Diplomonadida, Trichomonadida, and Oxymonadida) and many Sarcodina have one nucleus. Moreover, nearly all Sporozoa have uninucleate stages in their life cycles. There are, however, also multinucleate

forms with several identical nuclei in almost every group of protozoans. These forms are usually larger than their uninucleate relatives. In such cases, *nuclear polymerization* occurs.

In many protozoans with a complex life cycle, uninucleate stages alternate with multinucleate ones. In Coccidia, for instance, sporozoites and merozoites are uninucleate, while schizonts and microgametocytes are multinucleate. In such cases, multinuclearity is a temporary phenomenon related to the fact that nuclear divisions surpass cytoplasmic ones. The multinuclearity of some stages of the life cycle is more stable in the Foraminiferida: the specimens of the haploid sexual generation (the gamonts) are often uninucleate, while the specimens of the diploid asexual generation (the agamonts) are multinucleate.

In multinucleate protozoans, all the nuclei are usually morphologically alike and capable of undergoing mitosis. This applies, for instance, to the multinucleate amoebae *Chaos* and *Pelomyxa*, the heliozoan *Actinosphaerium*, and the flagellates of the subphylum Opalinata. Such protozoans are said to be *homokaryotic*. But the nuclei can greatly differ from each other forming two categories—*generative* and *somatic* nuclei. The former are usually small and divide by mitosis, while the latter are large and either do not divide at all or divide by direct pinching in two or by budding, which externally resembles amitosis. Both types of nuclei, however, have a common origin. In this case, *nuclear differentiation* or *nuclear dualism* occurs and the protozoans involved are said to be *heterokaryotic*. The most widely known examples of heterokaryotic protozoans are ciliates in which the generative (small) nucleus is called the *micronucleus*, while the somatic (large) nucleus is called the *macronucleus*. Nuclear dualism in ciliates and other protozoans will be discussed in detail in Chapters 7 and 8.

The shape of protozoan nuclei is very diverse. Spherical and ellipsoidal nuclei are found most frequently (Fig. 1), while spindle-shaped, cap-shaped, elongated, lobulated, and other nuclei are seen more rarely. The nucleus of *Amoeba proteus* is disc-shaped (Fig. 4c) while that of the radiolarian *Thalassophysa* has sacculiform pockets on the surface (Fig. 66). The macronuclei of ciliates have the most diverse shapes. They can be sausage-shaped, moniliform, branched, etc. (Fig. 90).

The dimensions of protozoan nuclei vary greatly. Nuclei with a diameter of about  $1\ \mu\text{m}$  and even less occur in *minute* intracellular parasites: the flagellates *Leishmania*, malaria parasites, etc. The diameter of the nucleus of the smallest free-living protozoans (e.g. the flagellates *Pedinomonas*, *Heteromastix*, and *Sphaleromantis*) is also within the range of  $1\text{--}2\ \mu\text{m}$ . The micronuclei of ciliates are also small; they range from  $1\ \mu\text{m}$  in *Blepharisma wardsi* to  $7\text{--}8\ \mu\text{m}$  (rarely to  $20\ \mu\text{m}$ ) in *Paramecium bursaria*. The largest spherical protozoan nuclei have a diameter of  $400\ \mu\text{m}$  (in the radiolarians *Planktonetta atlantica* and *Thalassophysa sanguinolenta*) and even  $1\ \text{mm}$  (in *Thalassophysa melacapsa*; HOLLANDE and ENJUMET 1953). Thus, the diameter of the largest protozoan nuclei is 1000 times greater than that of the smallest ones, while the volume of the largest ones is  $1000^3$ , i.e. a billion times greater than that of the smallest ones! The macronuclei of some ciliates (e.g. *Bursaria*) are also very large, but they usually have a complex shape; therefore, it is difficult to compare their volume with that of the other nuclei. It should be noted that usually the largest protozoan nuclei either are polyploid, i.e. consist of several identical chromosome sets (radiolarian nuclei, macronuclei of some ciliates) or

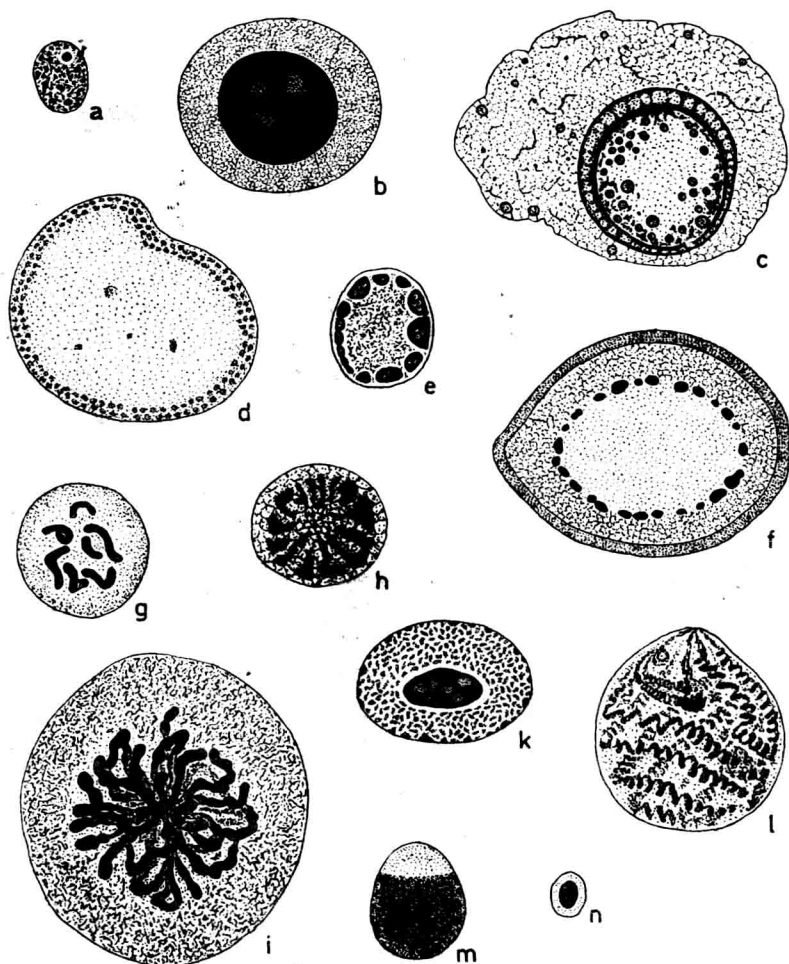


Fig. 1. Main types of nonpolyploid nuclei in protozoans. *a* *Trichomonas muris*, 2500 $\times$ ; *b* *Amoeba sphaeronucleolus*, 1500 $\times$ ; *c* *Aggregata eberthi*, a macrogamete, 1000 $\times$ ; *d* *Amoeba crystalligera*, 1500 $\times$ ; *e* *Amoeba terricola*, 1500 $\times$ ; *f* *Endamoeba blattae*, 1500 $\times$ ; *g* *Euglypha* sp. (sausage-shaped nucleoli), 1000 $\times$ ; *h* *Actinosphaerium eichhorni* (rosette-like arrangement of nucleoli), 1450 $\times$ ; *i* *Duboscquella aspida* (a branched central nucleolus), 1200 $\times$ ; *k* *Euglena* sp., 1200 $\times$ ; *l* *Holomastigotoides psammotermittidis*, 1500 $\times$ ; *m* micro-nucleus of *Paramecium caudatum*, 1500 $\times$ ; *n* micronucleus of *Paramecium aurelia*, 1500 $\times$ . *a-h*, *k*, *m*, and *n* after BĚLAŘ (1926a); *i* after CACHON (1964); *l* after GRASSÉ and HOLLANDE (1963).

have many isolated gene copies that are selectively multiplied as a result of a process that resembles gene amplification (macronuclei of other ciliates). Such nuclei will be considered in detail in Chapters 6 and 8.

## Structural Components of Nuclei

### Nuclear Envelope

**Typical Structure.** All protozoan nuclei are surrounded by a nuclear envelope. Basically, this envelope has the same structure as that of the cell nuclei of other eukaryotes (FRANKE and SCHEER 1974), i.e. it consists of two parallel unit membranes (outer and inner ones), each of which is about 7.5 nm thick and separated from the other by perinuclear space. The nuclear envelope is perforated with pores at whose periphery the outer nuclear membrane is continuous with the inner one (Fig. 2a, f, g). In sections that are tangential to the nuclear surface, the pores usually have the form of dense rings or annuli (Fig. 2b, c), while the central granules can be seen in the pore openings. The annulus is formed by a ring of dense material along the pore edges. The nuclear pore with the accompanying structures (annulus, central granule, etc.) is called the pore complex. The inner surface of the nuclear envelope is often covered with a thin layer of amorphous or indistinctly fibrillar material called fibrous lamina.

This classic structure of the nuclear envelope has been observed in many eukaryotic Protozoa belonging to all protozoan phyla (see the column Nuclear Envelope in Table 1). Among the Sarcodina, however, the nuclear envelope is of a typical structure only in forms with rather small nuclei. As a rule, the nuclear envelope of sarcodinians with large nuclei (i.e. foraminiferans, radiolarians and large uninucleate amoebae) has various complications. Among the Sporozoea, the nuclei of trophozoites of only some gregarines have a "classic" envelope (Fig. 2a); this envelope is more complex among other Gregarina and occasionally in the gamonts of the same species (Table 1). As for the Ciliophora, their generative nuclei (micronuclei) as well as their somatic nuclei (macronuclei) are invariably surrounded by a classic two-membrane envelope in which more or less frequent pores are regularly seen (Tables 1 and 6).

**Pore Complexes.** The number of pore complexes per unit surface of the protozoan nucleus varies greatly. In general, it is proportional to the size of the nucleus: as a rule, there are many more pores per square  $\mu\text{m}$  on the surface of large nuclei than in the same area on the surface of small nuclei. Very sparse pores are characteristic of the nuclei of some flagellates, i.e. the chrysomonadid *Pseudodendromonas* and the cryptomonadid *Cyathomonas* (MIGNOT 1965, 1974a), the nuclei of the merozoites of the gregarine *Selenidium* (SCHRÉVEL 1971b), the nuclei of the trophozoites of avian malaria parasites (AIKAWA 1966), etc. Pores are rather sparse in the envelope of the micronucleus of the ciliate *Nassula ornata*, numbering 12 per square  $\mu\text{m}$ , while they number more than 80 per square  $\mu\text{m}$  in the envelope of the macronucleus (RAIKOV 1966). There are, however, 30–40 pores (RAIKOV and DRAGESCO 1969) and even 60 pores per square  $\mu\text{m}$  (RAIKOV and KOVALEVA 1978) in the micronuclei of the karyorelictids belonging to the genus *Tracheloraphis*. A moderate pore frequency (13–15 per square  $\mu\text{m}$ ) has been observed in rather small nuclei (6  $\mu\text{m}$  in diameter) of the rhizopod *Gromia* (HEDLEY and WAKEFIELD 1969).

The nucleus of the trophozoite of the protococcidian *Coelotropha* has about 20 pores per square  $\mu\text{m}$  (VIVIER and HENNERÉ 1965; Fig. 2b).

Considerably greater frequency of the nuclear pores (30–35 per square  $\mu\text{m}$ ) has been observed in the dysenteric amoeba *Entamoeba histolytica* (MARTINEZ-PALOMO *et al.* 1976). On the same area of the nucleus, there are about 40 pores in the multinucleate free-living amoebae of the genus *Chaos* and in *Pelomyxa palustris* (DANIELS and ROTH 1964, DANIELS *et al.* 1966, 1969), 50–60 pores in the flagellate *Hypotrichomonas acosta* (MATTERN *et al.* 1969), about 80 pores in the hypermastigid *Trichonympha* (GRIMSTONE 1959), and about 100 pores in the foraminiferan *Globigerina* (FEBVRE-CHEVALIER 1971). The pore frequency is not constant for a given species: in *Euglena* it depends on the cultivation temperature (LOTT *et al.* 1977), while in *Acanthamoeba* it sharply decreases during encystment (WILLAERT *et al.* 1978a).

In ciliates, the pores are very numerous on the envelopes of the macronuclei belonging to the diploid and especially to the polyploid type. As regards the former, one square  $\mu\text{m}$  of the surface of the nucleus has about 40 pores in *Tracheloraphis caudatus* (RAIKOV and DRAGESCO 1969), about 60 pores in *Loxodes magnus* (RAIKOV 1975a), and about 85 pores in *Tracheloraphis phoenicopterus* (RAIKOV and KOVÁLEVA 1978). As regards the latter, the figure comes to about 35 in *Chilodonella cucullulus* (RADZIKOWSKI 1973a), about 50 in *Neobursaridium gigas* (NILSSON 1969), about 75 in *Woodruffia metabolica* (GOLDER 1976; Fig. 2c), 60–80 in *Paramecium primaurelia* (STEVENSON and LLOYD 1971b), about 100 in *Didinium nasutum* (RIEDER 1971, KARADZHAN and RAIKOV 1977a), and up to 140 in *Nassula* sp. (TUCKER 1967). It has been shown that in *Tetrahymena pyriformis*, pore frequency depends on the physiological state of the ciliates: there are 70–145 pores in the stationary phase of culture growth and 180–190 pores in the logarithmic phase per square  $\mu\text{m}$  of the surface of the macronucleus (WUNDERLICH and FRANKE 1968, WUNDERLICH 1969a). The diameter of the pore openings is less in the logarithmic phase than in the stationary phase, being 47 and 56–65 nm, respectively. When the pores are very numerous, they are arranged on the nuclear surface in a regular order (usually hexagonally).

A thorough study of the structure of the pore complexes in some protozoans has shown that it is not always the same. As a rule, the *annulus* is revealed on the tangential sections of the nucleus not as a homogeneous ring, but as a crown of

(Continued on p. 38)

Fig. 2. Ultrastructure of the nuclear envelope and pore complexes in protozoans. *a* a part of the trophozoite nucleus of the gregarine *Selenidium* (1 nucleolus; 2 pores of the nuclear envelope), 30 000  $\times$ ; *b* pore complexes in a tangential section through the nuclear envelope of the coccidian *Coelotropha*, 70 000  $\times$ ; *c* same in the macronuclear envelope of *Woodruffia*, 56 000  $\times$ ; *d, e* tangential sections of pore complexes of the amoeba *Chaos carolinensis* (*d* 47 500  $\times$ ; *e* 160 000  $\times$ ); *f* same, a cross section (the arrows indicate the subunits of the annulus), 72 000  $\times$ ; *g* cross section of a pore complex of the gregarine *Selenidium* (the arrows indicate the annulus, the double arrow shows the central element), 100 000  $\times$ ; *h* honeycomb layer on the outer surface of the nuclear envelope in *Endamoeba blattae* (the arrows indicate pore complexes), 25 000  $\times$ . *a* after VIVIER, SCHRÉVEL (1964); *b* after VIVIER and HENNERÉ (1965); *c* after GOLDER (1976); *d, f* after DANIELS and others (1969); *g* after VIVIER (1967); *h* after BEAMS and others (1959).