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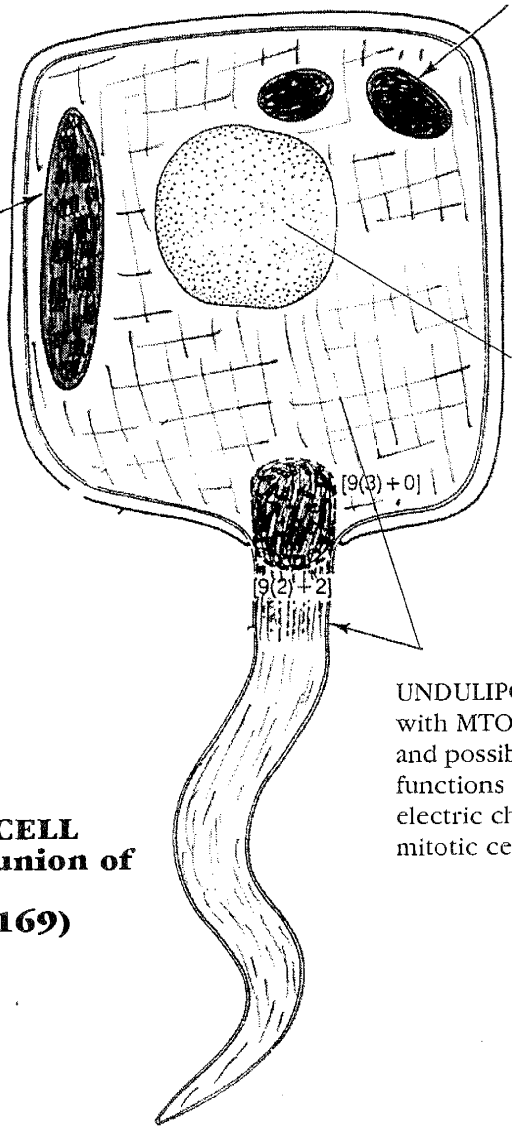
CELLS LIVING INSIDE CELLS: NEW UNDERSTANDING OF THE ORIGIN OF OTHER LIVING ORGANISMS FROM BACTERIA

John Coates



A EUKARYOTIC CELL interpreted as the union of four different cells (explanation on p. 169)

SUMMARY

Living cells are divided into two kinds: bacteria (prokaryotes), which are small and lack a clearly-defined nucleus to contain their genetic material DNA (DeoxyriboNucleicAcid), and eukaryotes, either single-celled or multicellular organisms, which are larger (typically about 50 μm in diameter rather than 1-4 μm), have their DNA in nuclei and differ from bacteria in many other ways, such as in having much larger pro-

tein molecules. A eukaryote cell has much more DNA than a bacterium and it is wrapped with histone protein in threadlike chromosomes.

Eukaryote cells have other structures (organelles) not found in bacteria. These include mitochondria which finish the breakdown of sugars with oxygen (respiration) in both plants and animals, and plastids (chloroplasts) in which algae and plants use chlorophyll to make sugars using water and carbon dioxide. It is now generally accepted that mitochondria and plastids are derived from bacteria which went to live inside other cells and soon became unable to live outside. An intimate association between unlike organisms from which both benefit is called symbiosis.

Other structures in eukaryote cells which may be bacterial in origin include the cytoskeleton which maintains and changes the shape of a cell, motility structures (cilia and flagella), and microtubules and their organising centres which also form spindles and other structures essential to the eukaryote mode of cell division called mitosis. If they are derived from bacterial symbionts, they have hidden their origins by losing all or nearly all of their DNA and characteristic structure but they retain the distinctive proteins, the three tubulins, of the spirochaete bacteria from which they seem to be derived. Tubulins are also vital to the function of nerve cells.

Evidence for our present understanding comes from study of both living organisms and fossils, and uses a wide variety of laboratory techniques. The story can conveniently be set out under four heads: what is the evidence that intracellular organelles are derived from bacteria, what were the first symbionts like, how did the union happen, and what have been the consequences?

STUDY OF LIVING ORGANISMS

There are many living examples of micro-organisms with bacteria living inside their cells and these com-

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CONTENTS

MICROSCOPICAL SOCIETY OF SOUTHERN CALIFORNIA

President- James D. Solliday 1130 S. Austin St. Santa Ana, CA 92704. 714-775-1575 jlsolliday@home.com.
Vice President - Ken Gregory, 2124 Ocana Ave. Long Beach, CA 90815. 562-596-1762.
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Facilities Chairman - Pierino Teti, 974 Edgecliffe Drive, Los Angeles, CA 90026. 323-660-9259.

Publication Correspondence To
Editor Gaylord E. Moss
P.O. Box 9130
Marina del Rey, CA 90295
Tel/FAX (310) 827-3983
gmoss@mediaone.net

Dues and Membership Applications To
Treasurer David L. Hirsch
11815 Indianapolis Street
Los Angeles, CA 90066-2046
Tel (310) 397-8357
dlhirsch@earthlink.net

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monly show that DNA has been exchanged between host and bacteria; inevitably this results in net loss of DNA by the bacteria, which become unable to live outside their host cells.

Pelomyxa palustris is an apparently simple unicellular organism without mitochondria or chloroplasts which lives on the bottom of a foul pond with virtually no free oxygen. Each individual contains hundreds of bacteria of at least three kinds, some on the membranes of its many nuclei and others distributed through the cytoplasm. *Pelomyxa* feeds normally but cannot respire with neither oxygen nor mitochondria: the host cell metabolises carbohydrates to lactic acid (as our muscles do when oxygen runs short) and its resident bacteria convert this to methane which is excreted. *Pelomyxa* has become dependent on its bacterial symbionts: if they are killed by treatment with an antibiotic, lactic acid accumulates in the host's cytoplasm and it dies soon afterwards.

The establishment of symbiosis between amoebae and bacteria has been observed. A culture of amoebae was found to be infected by pathogenic bacteria, but those amoebae which survived eventually reduced their bacterial population from 100,000 or more to about 40,000 per cell; but by then DNA had been exchanged between host and pathogens so that the amoebae could not live without their bacteria, which were no longer harmful but symbiotic partners. The loss of essential DNA from host nuclei was demonstrated once the partnership had stabilised by experiments involving exchange of nuclei between infected and uninfected individuals. Over time and especially with fewer endosymbionts there will inevitably be a net transfer of DNA from them to the nucleus, since daughter amoebae without essential DNA are not viable but loss of DNA by individual bacteria in the cell is not serious because copies will remain in the nucleus and other bacteria.

Suggestions that normal organelles in eukaryotic cells were bacteria were first made soon after use of the compound microscope became common in the 1880s. Good observational comparisons between chloroplasts and Cyanobacteria (then called blue-green algae), and between mitochondria and what we now call purple bacteria, were made by several workers up to the 1920s. The organelles were typically the size and shape of the corresponding bacteria, reproduced independently of the cell nucleus, and so on. The Russian botanist Konstantin S. Merezhkovsky (working 1903-10) coined the term *symbiogenesis* for the origin of new organisms by the genetic union of different ones rather than by progressive genetic change in a single species. Some early experimentalists thought they could culture organelles apart from their host cells as they could bacteria, but did not realise that this was impossible because the organelles had lost genetic material essential for independent life; when they claimed success the bacteria they grew were presumably contaminants.

Modern understanding began in 1962 when chloroplasts of the unicellular alga *Chlamydomonas* were found to contain structures like bacterial DNA. This was only a few years after Watson and Crick's pioneer work on DNA, and biologists were thinking of nuclear DNA as controlling everything that went on in a cell, so the idea of DNA elsewhere and so unlike that in the nucleus was not readily accepted. Then in 1967 Lynn Margulis (then Sagan), who was largely unaware of the earlier Russian work, proposed that mitochondria, chloroplasts and motility organelles were symbiotic in origin: this was the extreme symbiogenesis theory, and it stimulated modern study, if only in attempts to disprove it.

THE ORIGIN OF MITOCHONDRIA

(i) Evidence for their bacterial nature: attempts to relate mitochondria to other structures in cells, and especially the membrane system in the cytoplasm, have all failed. No cells are known with incompletely-formed mitochondria or structures intermediate between them and anything else such as golgi apparatus. They are either present and complete, or absent. They resemble free-living bacteria in structure, biochemistry and genetics. They have a little DNA, which is like that of bacteria and unlike that in the nucleus; it not associated with histone proteins like that of eukaryote chromosomes. Mitochondria divide independently of the nucleus and when they do their DNA replicates so that each daughter mitochondrion has a complete copy. Their genetics is separate from that of the nucleus but they readily exchange copies of genes with each other and with the nucleus so far as this is possible with the few genes they still have. They have their own ribosomes of eubacterial type containing RNA (RiboNucleic Acid) on their own internal membrane system and making their own proteins.

(ii) What were the first symbionts like? Virtually all eukaryotes have mitochondria, and there is evidence that those few microbes without have either lost them or have similar organelles with related functions, so the acquisition of mitochondria was close to the origin of eukaryotes from bacteria.

Bacteria are divided into two groups. **Eubacteria**, the great majority of known forms, have tough outer walls, their ribosomes are less like those of eukaryotes, and they are found in a great variety of habitats. **Archaebacteria or Archaea** lack a tough capsule outside the cell membrane and they resemble the simplest eukaryotes in several significant ways: at least some have DNA wrapped in histone-like proteins, their gene regulation and transcription, and several distinctive proteins and enzyme pathways; their ribosomes are like those of eukaryotes and they show the begin-

nings of an internal membrane system like that of eukaryote cells. Some live in hot, anaerobic, often strongly acid places like the hot springs of Yellowstone, but in recent years many have been found in more conventional environments such as sea water, lakes and soils. It was formerly thought that they resemble the earliest bacteria because their habitats resemble those of the young Earth, but it now seems that they arose from Eubacteria by loss of the cell wall. The host cell was either an archaebacterium or an early eukaryote differing most obviously in having its DNA wrapped in a nucleus. If it was an archaebacterium like those living now, it would not have been able to ingest solid particles because it lacked the cytoplasmic motility machinery of eukaryotes.

The acquisition of mitochondria may have happened only once in the history of life, or at most three times. The living Eubacteria most like the presumed ancestor of mitochondria are the purple bacteria, and biochemical studies have concentrated on *Paracoccus denitrificans* which functions in some ways remarkably like a mitochondrion except that it has a full genetic makeup and is free-living. It can both photosynthesise, although not using chlorophyll and not well, and respire. Its RNA is more like that of mitochondria than that of any other bacterium known and it has more parts of the chemical machinery of mitochondria used in the same ways than any other bacterium which has been studied. This does not, of course, suggest that the living *Paracoccus* is a close relative of the bacterial ancestor billions of years ago, but it does confirm that a bacterium can have a biochemical makeup very like that which the ancestor is thought to have had.

(iii) How did the symbiosis come about? This question contains two others: what advantage was the association, and how did a eubacterium enter an archaebacterium or an early eukaryote which may not have been able to ingest it?

Life arose when Earth's atmosphere contained hardly any free oxygen, a gas which was and is highly toxic to living material: it denatures proteins and nucleic acids, for example. Air was rich in hydrogen sulphide, and some eubacteria used it as a raw material for building sugars: a molecule of hydrogen sulphide is readily broken to yield hydrogen ions and a pair of electrons, both used in the process, and waste sulphur. Volcanic activity was the chief source of hydrogen sulphide: when this decreased and more bacteria came to use the gas the supply was depleted. Eventually some bacteria modified a cytochrome molecule already used in electron-transport systems and produced chlorophyll which enabled them to use water as a raw material instead. These were the Cyanobacteria, very like their descendants living now, and their activity produced waste oxygen which accumulated in the air.

An association of a purple bacterium and an archaebacterium would have benefitted the partners in either of two ways. (a) The eubacterium might have protected the latter from toxic oxygen. If the host was an archaebacterium living in acid, partly its own waste, an associated purple bacterium could oxidise that to carbon dioxide and water; in such a culture medium, adding a bacterium to metabolise the acid enhances the growth of the archaebacterium. (b) In many environments Archaebacteria make sugars from hydrogen and carbon dioxide and produce methane waste; it has been suggested that the purple was a fermenter producing hydrogen and carbon dioxide which its partner could use.

With oxygen available, the purple bacterium could exploit it by completing the respiratory process and thus becoming mitochondria as we know them, so making water instead of hydrogen waste, carbon dioxide and far more ATP (Adenosine TriPhosphate) than before: this is the molecule used by both bacteria and eukaryotes to drive energy-consuming processes. The vast majority of eukaryote cells ever since have used mitochondria to consume oxygen and generate ATP abundantly from it. A few microbes like *Pelomyxa* have taken to anaerobic habitats and lost mitochondria but have symbiotic bacteria to consume the lactic acid they produce; before this happened, however, some of their genes were lost to the host nucleus and they give us a clue to the sequence of events. Other microbes (such as those ciliates which live in the intestines of animals) have apparently converted their mitochondria to organelles called hydrogenosomes, which without oxygen produce not water but waste hydrogen.

How did the eubacterium get inside its partner? Study of living bacteria suggests the answer. Bacteriologists now know many Eubacteria named as single species but which are associations of two kinds each supplying the other's needs for chemicals or energy and unable to live separately. Some are merely mixed in the same medium, with chemicals diffusing through the liquid between them, and others are actually in contact, one or several bacteria of one kind firmly attached to a different one. Contact minimises loss of useful substances, and in a close association between a **eu**bacterium and an **archae**bacterium it is easy to see that the latter would be likely to spread round the eubacterium to maximise the contact surface until the eubacterium was enclosed. This process would be much easier if one of the symbionts was not an archaebacterium like those we know but was an early eukaryote which could ingest solid particles, and we shall see later that this may well have been so.

(iv) What have been the consequences? The success of the symbiosis quickly led to diversification of

eukaryotes all with the same final pathway, respiration, for obtaining energy by breaking down sugars. The energy output is far greater than from non-aerobic processes such as fermentation and the waste products are carbon dioxide and water instead of acids and alcohols.

Eukaryote cells grew bigger, up to about 50 μm in diameter, with a big increase in nuclear DNA, and this enabled them to become more complicated, multicellular, and to exploit all possible aerobic environments. Mitochondria metabolising oxygen produced the first *steroid molecules* which helped stabilise the internal membrane system of a cell and made larger size possible. As far as we know, steroids occur naturally only in eukaryotes and the earliest rocks in which they are found are about 2,700 million years old, so that may roughly date the origin of eukaryotes.

Ever since, mitochondrial respiration has played a key part in *controlling the oxygen content* of air. Recently some eukaryotes have extended the concept of respiration in the traditional way of bacteria: we derive energy from the more-or-less controlled oxidation of various substances (wood, coal, petroleum products) which are not used as sources of body material. This produces heat and work far beyond the output of our own tissues, but again the composition of the atmosphere is changing as a result.

There are only two sexes. Many organisms, from microbes upwards, have mating types, and fusion of gametes or exchange of genetic material is always between cells identical except for their DNA. But wherever gametes are different, smaller swimming ones and larger waiting ones, there are only ever two mating types called male and female. Mitochondria are nearly always transmitted in female gametes only, and this seems to be because some mitochondria are "selfish": they contribute less ATP than others to the host cell but divide more often, and their indefinite spread at the expense of the others is prevented by transmission through eggs only.

How are things now? A typical mitochondrion in any eukaryote still looks like a purple bacterium. It lacks the tough outer capsule of a bacterium but still has the two-layered cell membrane. However, studies with the scanning electron microscope have shown that only the inner layer belongs to the bacterium: the outer layer is inside-out and studded with ribosomes of the host cell facing the host cytoplasm, so that this outer layer is evidently the host's replacement for the original which was lost with the bacterial capsule. The DNA content has been greatly reduced by loss of inessentials and transfer of genes to the host nucleus. Some remaining functions are shared with the nucleus, but it seems that a mitochondrion cannot lose more: either because some functions must stay where they are, or because mitochondria have departed from the universal genetic code and their DNA no longer codes for proteins as the host's DNA does, so that copying to the nucleus and loss by the mitochondrion would be fatal for the cell.

In 2000 the UK Forensic Science Service began profiling mitochondrial DNA, rather than nuclear DNA, from samples of human material in efforts to solve crimes committed many years before, and have already achieved success. The extreme sensitivity of the technique enables scientists to exploit the more distinctive character of mitochondrial DNA which has only a few genes to search. In one case a man was convicted after eighteen years partly on the identification of his mitochondrial DNA in samples of semen at the scene of rape and murder.

THE ACQUISITION OF CHLOROPLASTS (PLASTIDS)

There is no controversy about this: early eukaryotes with mitochondria ingested photosynthesising Eubacteria but some resisted digestion and made themselves at home. We ask the same questions as before.

(i) What is the evidence for their bacterial nature? They cannot be derived from other structures in a eukaryote cell but are either present and complete or absent. They are like free-living bacteria in structure, biochemistry and genetics. They have a little DNA, more than mitochondria, and it is like that of bacteria. They divide independently of the host cell and their DNA replicates so that each daughter cell receives a complete copy; their genetics, including recombination, is separate from that of the nucleus except when genes are exchanged between them. They have their own, small ribosomes of eubacterial type on their own internal membrane system. The various forms of RNA derived from their DNA are like those of bacteria and their proteins have fairly small molecules. Balance between symbionts, rather than growth and metabolism of a single cell, is also suggested by the host's general housekeeping: surplus chloroplasts are digested, any dead or moribund ones are cleared away and growth of healthy ones is controlled by limiting supply of nutrients or by use of specific chemical growth inhibitors.

(ii) What were the first symbionts like? The hosts were eukaryotes with mitochondria; the endosymbionts were like modern Cyanobacteria, and there is clear evidence from fossils that these were in existence well before the first eukaryotes. The detailed mechanism of photosynthesis in all plants, algae and other eukaryotes with chloroplasts is still astonishingly like that of Cyanobacteria.

(iii) How did the symbiosis come about? Green bacteria were simply ingested for food. One recent book lists eight different acquisitions from the remote

past until recently, but more are happening. The reader may have met a green *Paramecium* which has green bacteria as new symbionts: it was claimed in the 1940s that these could still be cultured outside the host.

Chloroplasts liberating oxygen into host cytoplasm would have killed the host without mitochondria to consume it, but no eukaryote with chloroplasts lacks mitochondria. The combination was successful: carbon dioxide from mitochondria supercharged photosynthesis which produced ample food for the host, oxygen from chloroplasts allowed rapid release of energy from sugars by mitochondria. The new symbionts were somewhat safer from other predators, protected from desiccation and had mobile homes for following the Sun, the source of the energy they used for photosynthesis. Microbes with chloroplasts are still mostly motile and aquatic.

(iv) What have been the consequences? Abundant food production by chloroplasts was a major benefit which led to genetic integration, but chloroplasts retain more DNA than mitochondria have. Genetic coding for production of some large enzyme molecules is shared with the nucleus, and this keeps the relationship in balance with neither able to outgrow the other. Genes have also been exchanged between chloroplasts and mitochondria: in maize they have a sequence of about sixty genes in common.

Chloroplasts have the internal structure of Cyanobacteria but pictures of it suggest electrical connections designed to run at a higher voltage. The number of outer membranes may be two, the inner one from the ingested bacterium and the outer, inside-out, from the host, three (from ingestion of a chloroplast, with an extra outer membrane from the new host) or four (from ingestion of a unicellular alga with a chloroplast), clues being which way out each layer is and whether the ribosomes attached to it are of eubacterial or eukaryotic type.

The abundance of organic food provided by photosynthesising eukaryotes was essential to the origin of multicellular animals feeding on them. When plants first colonised dry land about four hundred million years ago they were soon followed by animals and ever since plants of all sizes have supported other life at sea and on land. Lynn Margulis wrote:

"Today, locked inside every plant and many protists (such as the mobile *Euglena*), plastids ply the biosphere with food and oxygen - a far greater contribution than any made by the world's entire population of mammals. Plastids make food from water and sunlight. Mammals - including humans, of course - do no such thing. From a planetary point of view, the major role of mammals may be as fertilizers of plants and carriers of mitochondria. But if all mammals were to die in one instant, insects, birds, and other organisms would carry mitochondria and fertilize plants. If plants with their plastids were to suddenly disappear, however, the output of food on the planet would be so severely hampered that all mammals would certainly die."

ARE MOTILITY ORGANELLES DERIVED FROM SYMBIOTIC BACTERIA?

This would be the subject of vigorous controversy if only most authors did not ignore it. Protagonists claim that cytoskeletal and motility structures in eukaryotes are so common and so alike that they show a common origin which must therefore be bacterial, but it is usually difficult or impossible to demonstrate any remaining DNA outside the cell nucleus, let alone show that it is bacterial, and bacterial structures outside the nucleus have been so transformed that their origin cannot be proved. There is however evidence of sorts, including the use in movement of distinctive proteins, the three tubulins, which eukaryotes share with one remarkable eubacterial group, the spirochaetes, and observations of living spirochaetes which are used in movement of simple eukaryotes. If the structures claimed to be of spirochaetal origin really are, then spirochaetes must have been involved in the origin of eukaryotic cells and were soon so integrated that they lost both recognisable separate DNA and structure. As two recent authors said, the idea has not been disproved and we like it, so we are going to discuss it.

What structures are we talking about?

Cilia and flagella: Archaebacteria lack motility structures. A eubacterial flagellum is a stiff hair made of a single protein, flagellin; it is moved by what is called a rotary motor in the cell membrane and wall. The cilia and flagella of eukaryote cells, which neologophiles call undulipodia, have been shown by transmission electron microscopy to have the same structure in all eukaryotes which have them and it is unlike that of bacterial flagella. They are built on a similar arrangement of microtubules, long hollow threads usually grouped as nine or so pairs round a central pair. There is nothing magic about the nearly-universal number nine in this pattern: given the diameter of a microtubule set by its protein structure and the diameter of the shaft suitable for the flexibility needed, nine will usually be the number of microtubules which will fit into it. The microtubules are always made of the same proteins, the alpha-and beta-tubulins, with other proteins such as dynein and nexin; altogether the structure may involve two hundred or so different proteins. The shaft bends when the proteins pump sodium and potassium ions in and out, thus setting up electrostatic charges which move the microtubules against each other as if they were electromagnets in a solenoid arrangement. At the base of each hair the central pair of

microtubules disappears, the nine pairs of microtubules in the ring become three threes, and they surround a tiny region called the kinetosome or microtubule organising centre MTOC, which remains the subject of controversy. Microtubules usually grow from MTOCs, which contain the third tubulin, gamma-tubulin, which is believed to anchor the ends of microtubules and do much of the organising of structure and function. MTOCs seem to have almost the same structure throughout eukaryote cells.

Other microtubules: (i) The cytoplasm of a eukaryote cell contains a framework of microtubules, the cytoskeleton, which maintains and changes the shape of the cell and is responsible for the orderly movement of organelles such as chloroplasts around the cell.

(ii) Microtubules form the spindle which pulls chromosomes apart in the standard method of cell division called mitosis. Each chromosome has an attachment point, the centromere or kinetochore, which hooks on to a pair of microtubules, and when the chromosome divides the centromere becomes two so that the spindle microtubules can pull them to opposite ends of the cell. The MTOCs are the centrioles or centrosomes at the ends of the spindle, one where each daughter nucleus will be. A centriole has the same formation of a ring of nine triplets of microtubules as the base of a cilium or flagellum.

Cells with cilia or flagella withdraw them before cell division, and no cell is known to have them during division. (Ciliates like *Paramecium* are the exception, but they do not use spindles for nuclear division.) It is thought that microtubules used for cell movement are drawn in to be used during cell division, that the MTOCs divide just before mitosis so that each daughter cell will have a full set, and that afterwards they return to the cell surface and initiate growth of new cilia or flagella. The relationship between centrioles and MTOCs was first inferred, rather than observed, in 1924 by the Russian botanist B.M. Kozo-Polyansky in flagellated microbes such as trypanosomes; his observations could not be confirmed without electron microscopy. Most eukaryotes are multicellular and have special reproductive cells, so they do not have to give up movement while reproducing.

(iii) The fibres of animal nerve cells are bundles of microtubules, arranged differently but with the same proteins and dimensions as everywhere else; alpha-and beta-tubulins are the most abundant water-soluble proteins in our brains. Cross-sections of the the rods and cones in the light-sensitive retinas of our eyes show the standard pattern of microtubules. This may seem like a dramatic change of function, but microtubules work as ion pumps, creating electrostatic charges between one and another. If the fibres can move, as in cilia and flagella, curvature results. With some reorganisation of the fine structure and electrical insulation, movement is replaced by transmission of electrical charges, in other words the passage of nerve impulses.

The evidence for symbiosis

We can ask the same four questions as before about the presumed symbiosis, but if that is what it is then the bacterial symbiont has lost its identity so far that with our present knowledge the answers must be less satisfactory than for mitochondria and chloroplasts.

(i) What is the evidence for bacterial origin? There have been several attempts to demonstrate bacterial DNA associated with MTOCs, and these have mostly involved immunochemical fluorescence reactions, but the results have been difficult to repeat and have not convinced doubters so there is a lull while even more sensitive techniques are devised and made reliable. It is known that MTOCs have RNA, apparently combined with protein and hence called ribonucleoprotein RNP; this may be able to construct and replicate motility organelles under command from DNA in the nucleus without there being DNA in the MTOCs too. Whether this RNA differs from that in eukaryote ribosomes is not yet established. There is other evidence for non-nuclear DNA and three examples may be quoted:

Cortical mutations in some ciliates: The cortex of a ciliate like *Paramecium* is the surface layer of the cell, rich in cilia and MTOCs, and it is independent of the nucleus in some ways including repair or adaptation to wounds. Wounds arise when conjugating partners do not separate properly after exchanging micronuclei: either part of one is grafted on the other or they stick together permanently. Either abnormality is transmitted unchanged through as many generations as the observer wants to follow. Similar grafting oddities, such as surgical removal of a patch of cortex and replacing it the wrong way round, are also maintained for many generations. Healing and subsequent division involve replication of MTOCs and associated structures in the affected areas, but not nuclear DNA. If all the genes concerned were in the nucleus, one would expect things to be corrected soon or at the next cell division, so this is claimed as evidence of separate genomes for the cortical MTOCs. There are fascinating variations on this state of affairs in other ciliates: some can rectify cortical abnormalities, and this is attributed to interaction between nuclear DNA and the remnants of genome in the MTOCs.

Inheritance of cilia in some ciliates such as *Stentor* is independent of the nucleus; and in the gametes of several other protists growth and development of MTOCs and flagella continue in the absence of a nucleus, so that nuclear genes cannot be responsible for it.

The unicellular alga Chlamydomonas has a separate group of genes associated with each flagellum. It was first noticed from a mutant form with only one flagellum instead of the usual two: curiously, always the one further from the eye spot. The mutant gene, called "uni" from the effect of the mutation, was shown to be one of a group of about a dozen linked genes; the cell has two of these groups, and mutation of the uni gene in one of them leads to loss of its flagellum. Inheritance is normal, as though the two uni groups were a pair of small chromosomes, but studies of the results of breeding *Chlamydomonas* with different combinations of characters show that the genes in these groups are not joined in a linear chain like a eukaryote chromosome but are in a closed ring, the characteristic arrangement of bacteria. Nor are they in the nucleus: if they were, we would expect the uni mutation to affect both flagella equally. Each group is either in or on the visible MTOC at the base of a flagellum and its main function is to encode products used by the MTOC enabling its microtubule structure to work. Nuclear genes control development of the central pair of microtubules in each flagellum, and these may be genes which have been lost by the original symbiont to the nucleus. The paired uni groups look like two separate genomes, but attempts to locate them by an immunochemical test for DNA have not convinced other workers, who claimed that the colouring of MTOCs might be contamination by DNA from the nucleus: if this were so, it is amazing that contamination should reach only those two spots.

The three tubulins are found in all eukaryotes and in bacteria only in one group, the spirochaetes. Despite their varied functions in eukaryotes, they have hardly changed at all since the origin of eukaryotes and the three remain more distinct from each other than anyone of them has changed since. This is at least suggestive evidence that the tubulins of eukaryotes are derived from spirochaetes. MTOCs and related structures in eukaryote cells are distinctive and consistent in structure and function, and cannot be derived from other membranes or organelles.

(ii) What were the first symbionts like? One was either an archaebacterium or, more likely, an early eukaryote (with a nucleus) before symbiosis with purple bacteria established mitochondria; indeed, being able to ingest solid particles would have helped that to happen.

The other symbiont could only have been a spirochaete or something very like it. Some spirochaetes are associated with diseases such as syphilis, yaws and leptospirosis, and others are active predators which enter other microbes and swim around inside while feeding on their surroundings. Others live more conventional bacterial lives in sea or freshwater, deep muds or elsewhere. Seen with the low power of the microscope they are all much alike, coiled like corkscrews. They have bacterial flagella made of flagellin, some attached at one end and some at the other, between the two layers of cell membrane: their curving causes the sinuous swimming movement. Spirochaetes often associate with larger organisms for movement and food, sometimes casually but sometimes they have permanent relationships including jointly- constructed attachments. They have longitudinal microtubules which lie in the cytoplasm and contain tubulins. It is an intriguing problem that their own motility structures, the flagella, have not been transferred to eukaryotes which use the microtubules: presumably the answer will depend largely on the electrical properties of microtubules.

(iii) How did the symbiosis come about? Living microbes show what might have been an early stage in the association: many spirochaetes attached end-on to larger but simple eukaryotes and helping them move. The eukaryotes are parabasalids, like *Pelomyxa* in lacking mitochondria, which live in the intestines of termites and other insects. They ingest fragments of wood and other materials and contain bacteria which digest them, the insect getting some benefit from soluble nutrients produced. One parabasalid, *Mixotricha paradoxa*, has four flagella but these seem to be used only as rudders. Its surface is covered with an estimated half million spirochaetes of different kinds, attached end-on, most of which move synchronously like cilia and move *Mixotricha* much faster than it or they could move alone. As with chloroplasts, a major advantage of the association is exploiting motility. The host surface is sculpted into a pattern against which the spirochaetes fit their modified ends. It is not difficult to see that such an intimate and beneficial association could progress by permanent attachment of spirochaetes, followed by their conversion to cilia through simplification by loss of inessential structures, corresponding reduction of their DNA and transfer of the remnant partly to the host nucleus and partly to sites just below the cell surface where it would become MTOCs responsible for individual cilia.

(iv) What have been the consequences? Assuming that spirochaetes formed a symbiotic union with an early eukaryote, this probably would have happened before those with purple and green bacteria and very likely made the acquisition of mitochondria and chloroplasts possible.

That first association incorporated the three tubulins into the eukaryote cell and they became part of the common inheritance of all eukaryotes. They are remarkable multifunctional proteins which can self-assemble into microtubules in cells or even *in vitro*. Like most bacterial proteins they have fairly small molecules but alpha-and beta-tubulins join in pairs to

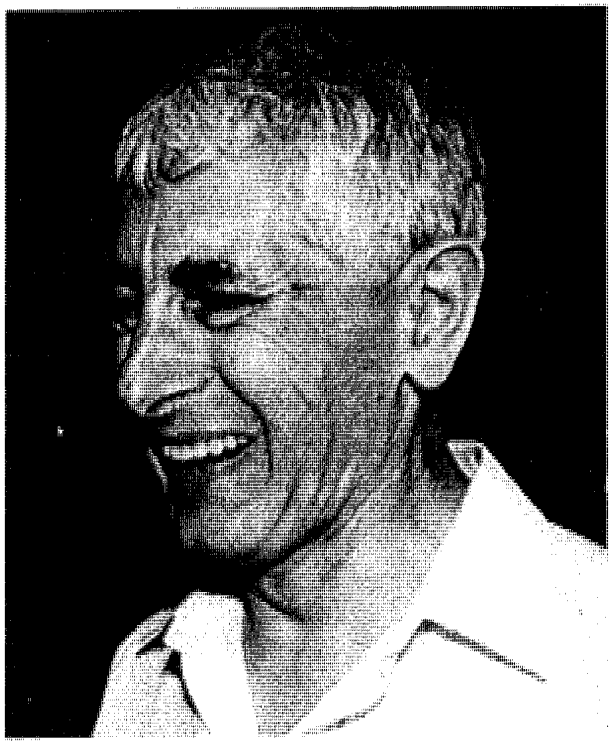
form the walls of microtubules. Microtubules readily bind to other proteins, resulting in the sophisticated cilia and other structures we see now. Benefits to the host would have included the construction of a cytoskeleton of tubulin and associated proteins, intracellular transport and movement of food vacuoles, vesicles containing digestive enzymes and so on including the efficient movement of chromosomes in cell division.

Some eukaryotes have tubulin-based structures but not cilia or flagella and sometimes not even cell division by mitosis. These include red algae and conjugating green algae, many amoebae and many obligate parasitic microbes. These may have lost external mobility organelles because they failed to use MTOCs for both motility and cell division.

Exploitation of tubulins has even allowed the establishment of conducting tissues in animals, leading to distinct nervous systems and sense organs. Here the commitment of tubulins to nerve conduction is such that fully-differentiated nerve cells cannot withdraw MTOCs from that function and so they cannot divide.

Again assuming that these ideas are right, we can now interpret a **typical eukaryotic cell** in a way which would have astonished scientists fifty years ago. *The nucleus* has DNA derived mainly from an archaebacterium, with contributions from two or three kinds of symbiotic bacterium, and is responsible for protein synthesis via RNA in ribosomes. *Motility organelles* such as cilia or flagella, microtubular structures of the cytoskeleton and centrioles have any DNA remaining outside the nucleus from the first partner, a spirochaete or perhaps some of them have RNA only. *Mitochondria* have the residue of DNA from a purple bacterium; they release most of the energy of sugars with oxygen by use of a special enzyme cycle peculiar to them. And lastly *chloroplasts* are derived from a cyanobacterial ancestor, most of its DNA having been lost or transferred to the nucleus; they are the photosynthetic machinery of algae, plants and other eukaryotes which have them. DNA has also been exchanged between at least two of the internal symbionts.

We have grown so used to the idea that the history of life is one of perpetual competition that it is truly astonishing to see that some crucial steps have been brought about by cooperation, even to the extent that the identities of partners may have been absorbed into the whole for the common good. But perhaps it is even more astonishing that after most of three billion years we can still clearly discern some of the characters involved and relate them to their unattached kindred.



John Coates

About the Author

John Coates trained as a zoologist, taking a PhD in agricultural science. He taught chemistry to university entrance level, then went into the pharmaceutical industry. From information support to a research team, he moved to export and transfer of technology to developing countries. Finally, he worked in a government sponsored body for simplifying international trade procedures and documentation. His business career culminated in international payment procedures. In retirement, he has been catching up with developments in biology.

Dr. J.I. Coates
21 William Smith Close
Cambridge Cambs England
sabrina@slewins.freeserve.co.uk

WORKSHOP of the Microscopical Society of Southern California

by: George G. Vitt, Jr.
Date: Saturday, 1 September 2001
Location: Ken Gregory's Residence

1. Jim Solliday introduced Herb Gold's guest, Marty Brenner, who is a balloon theodolite specialist, and who brought two such theodolites for display (see photos). He then outlined the steps that are being taken to get the publication of the Journal up to date. He added that MSSC has access to the Newroads School for workshops during our non-meeting times. Jim distributed the ordered copies of Ernie Ives' book on wood microtomy. He praised the Olympus Vanox microscope as being the 'last of the great scopes having the best optics, as tested by an independent lab.(see photos)

2. Pete Teti reported that, at this moment, there is no place available to hold the MSSC Christmas party.

3. Jim Solliday stated that he will be ordering some of John Welle's microslides and suggested that we need to hold a workshop on microtomy. George Vitt mentioned that a friend, who belonged to the Micological Society, had learned the technique of hand-holding mushroom samples and cutting thin sections with a straight razor.

4. Stuart Warter showed a McAllister microscope featuring a chain drive and a reverse foot (see photos).

5. Herb Gold showed a c.1980 Japanese made theodolite (see photo) made for tracking weather balloons for the purpose of determining wind velocity at various altitudes (see photos). Herb's guest displayed similar theodolites (see photos) of an earlier vintage. Herb also showed a rare British made 'Weather Balloon slide rule', cased, which was used to calculate weather balloon information. (This was packed up before a photo could be taken)..

6. Ken Miller showed his Swift microscope Mod. M400D where the eye piece diameter needs to be cut down in order that it fit his camera adapter Mod. PM-6.

7. John de Haas wanted to know if there was interest in his organizing a field trip to the Montclair CA area which is a source of micro-ruby mineral samples.

8. Jim Clark showed the book *Scientific American Book of Projects for the Amateur Scientist*, which he then presented to John Fedel.

9. Gary Legel brought, as freebies, many 35mm film plastic containers in which he had placed samples of diatomacious earth that he had gathered at Lompoc, CA. He also had his LOMO stereo microscope set up to show.

10. Alan de Haas described a relatively unknown procedure used by Leeuwenhoeck in fabricating his spherical glass lenses in order to reduce aberrations by making them aspheric. This he did by applying heat in one direction to the tiny spheres, causing the glass to distort from its previously spherical shape. Alan then showed an Aus Jena cased spindle stage kit which included the centerable stage, several eyepieces and infinity corrected strain-free objectives (see photo).

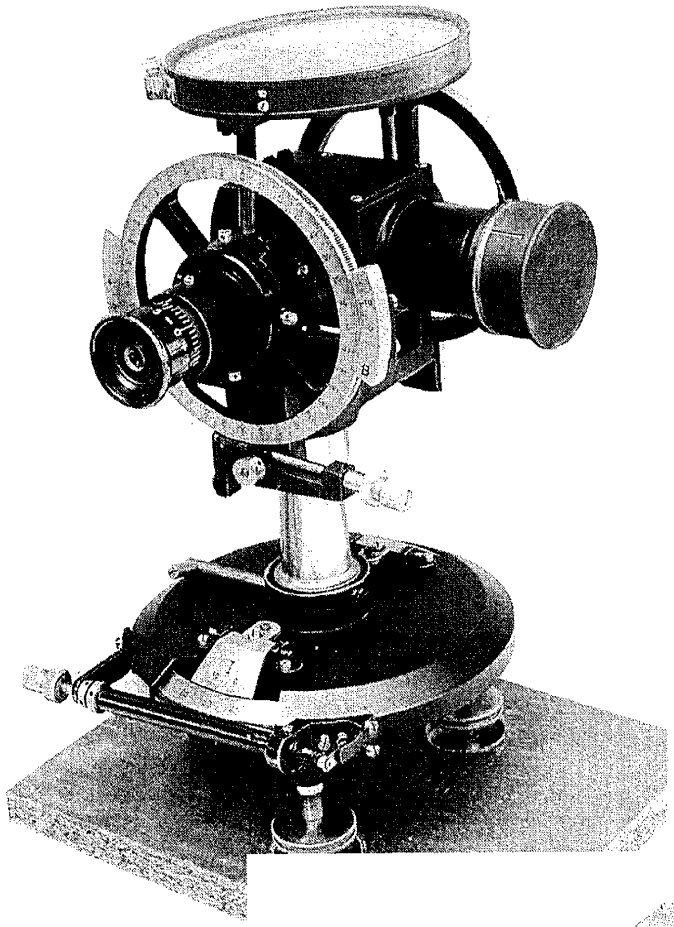
11. Gary Yaruss bought a Zeiss petrographic microscope with binocular tube and accessories.

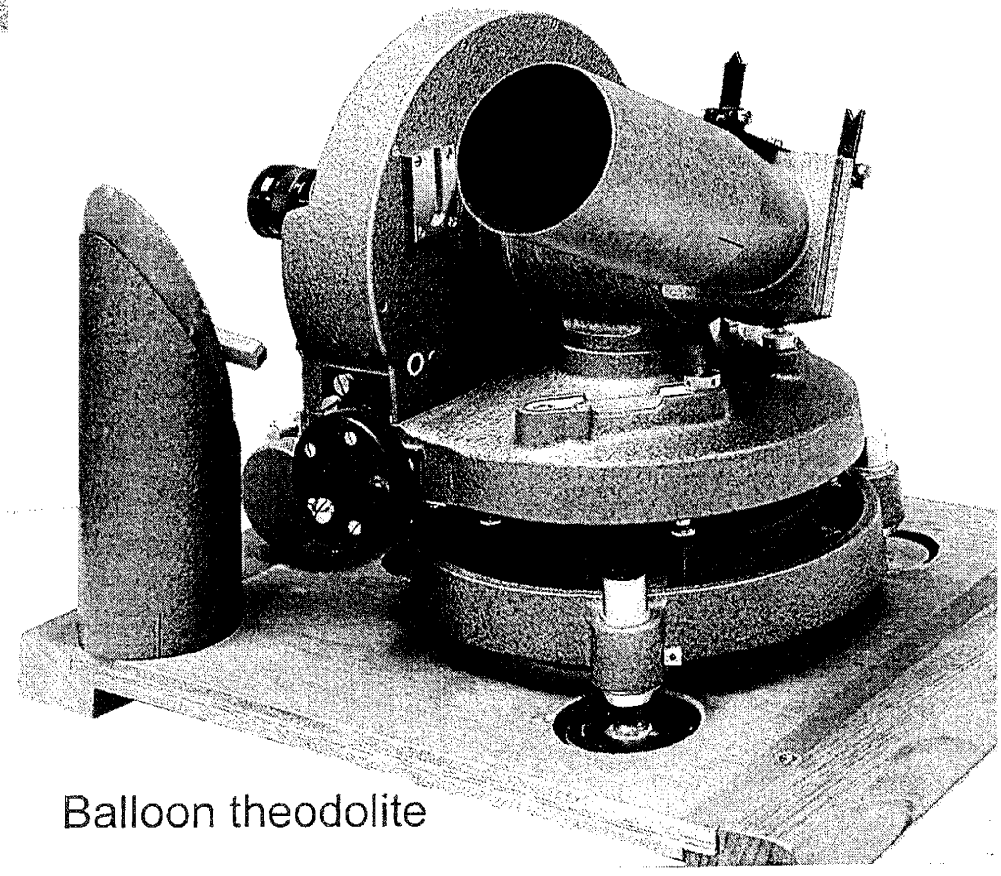
12. John Fedel showed some Zeiss Luminar objectives of Steve Craig's, which are for sale at $100 each. He stated that he will add to his Rocketry web site microscopy information and a link to panoramic photo stitching software..

13. Gaylord Moss announced that he will complete and mail the three issues of the MSSC Journal which will complete the set of volumes for the year 2000. He appealed to the members to write articles, noting that some 20 articles are needed for the coming issues for year 2001. Gaylord then showed and demonstrated his newly acquired laptop MacIntosh computer - the latest model built of Titanium and having all the bells and whistles of the latest technology and software.

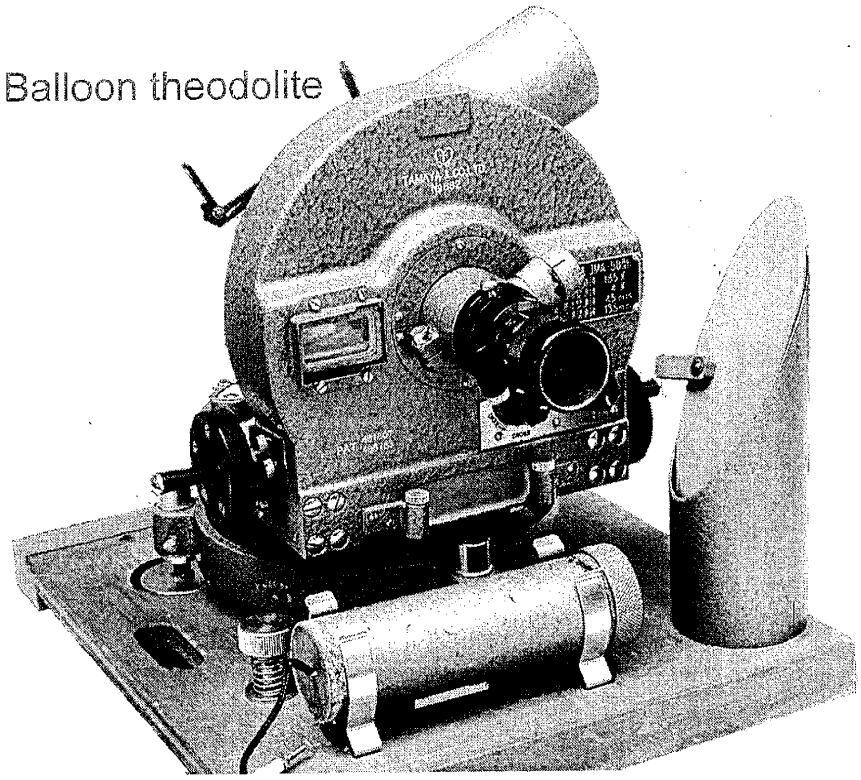
After the meeting, George Vitt took photographs of some of the items that had been exhibited. Members who wish to have their instruments photographed and published in the Journal should make sure that they are available for this purpose after the meeting, and should not pack them up prematurely and depart! In addition, I appeal to you all to provide me with a writeup, preferably by email, of the instruments exhibited so that they are adequately described in the Journal. My email address is gvitt@att.net. Thanks for your cooperation.

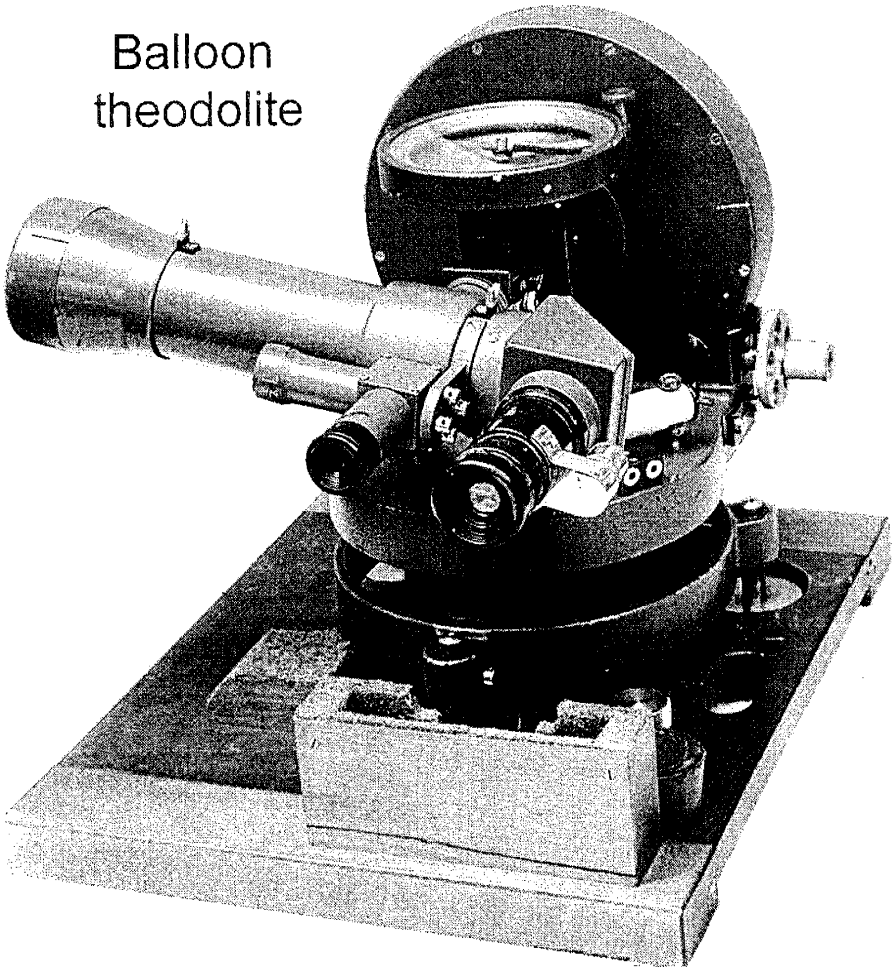
Balloon theodolite

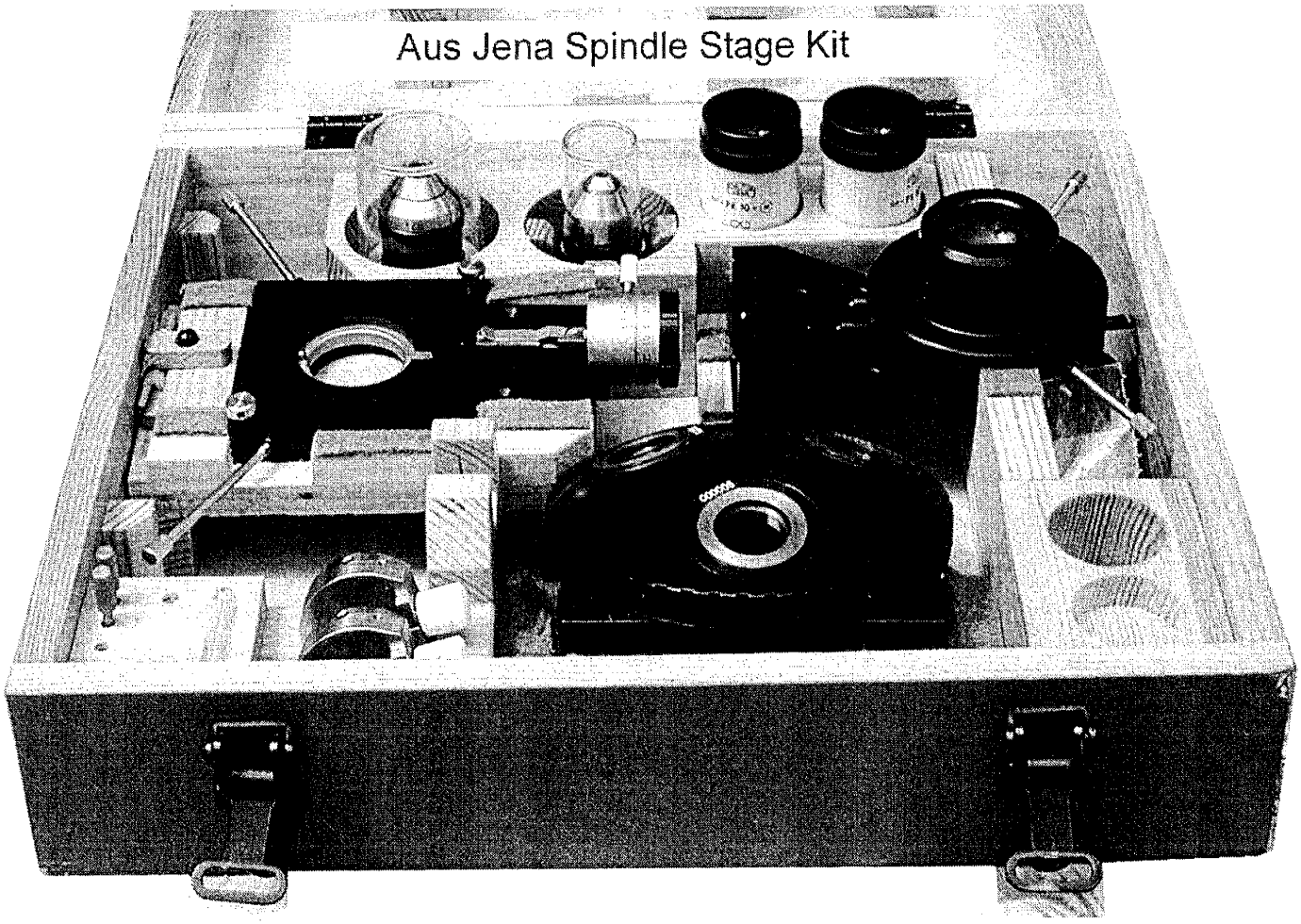


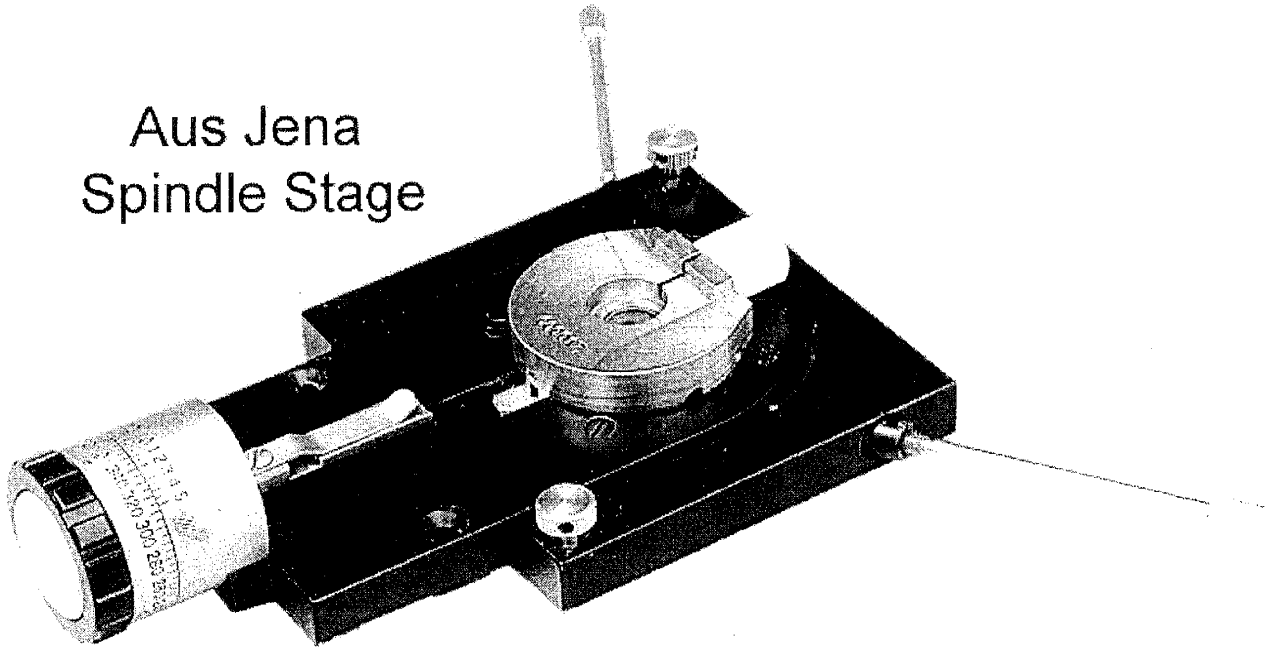


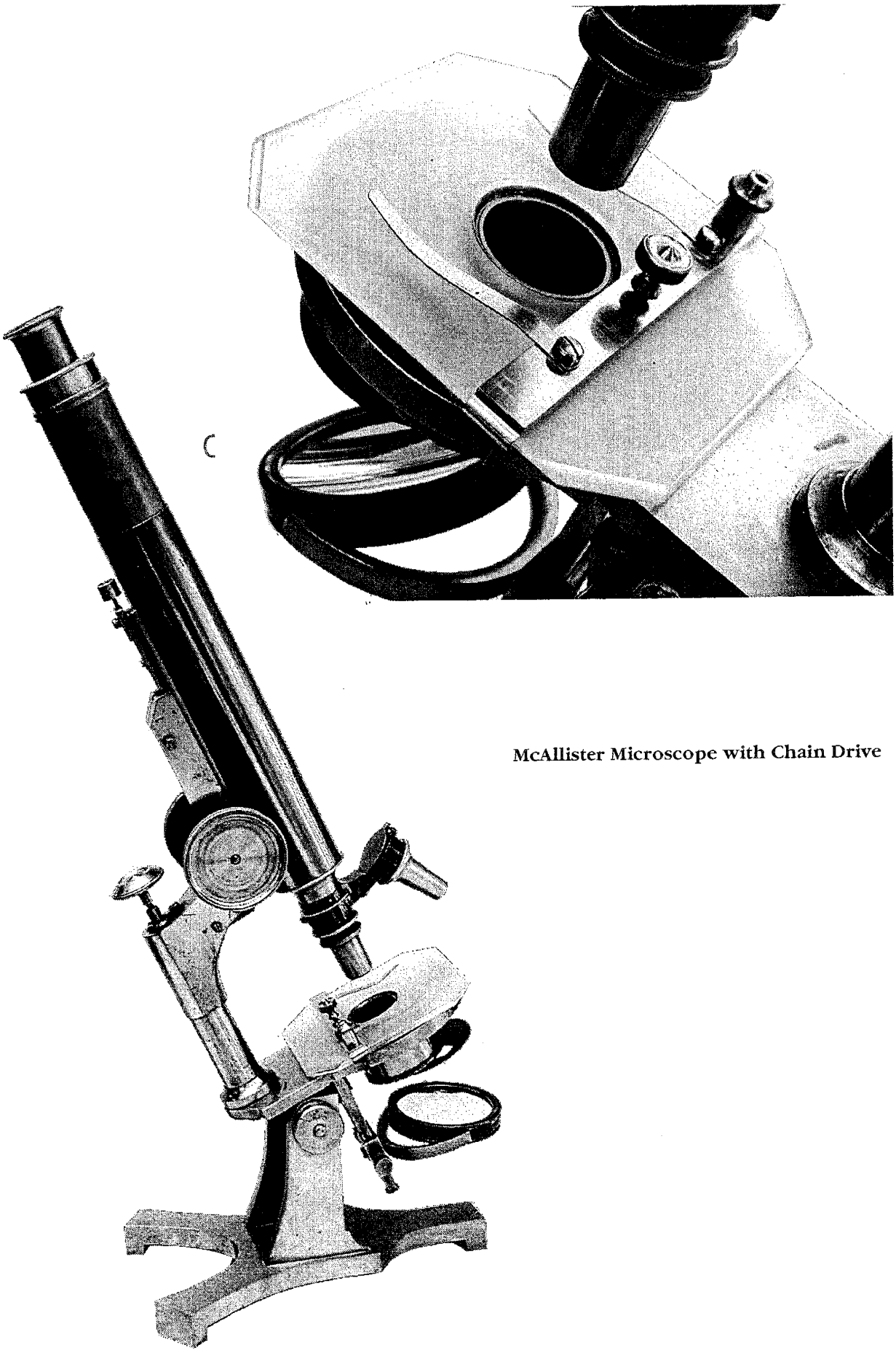
Balloon theodolite


Balloon theodolite

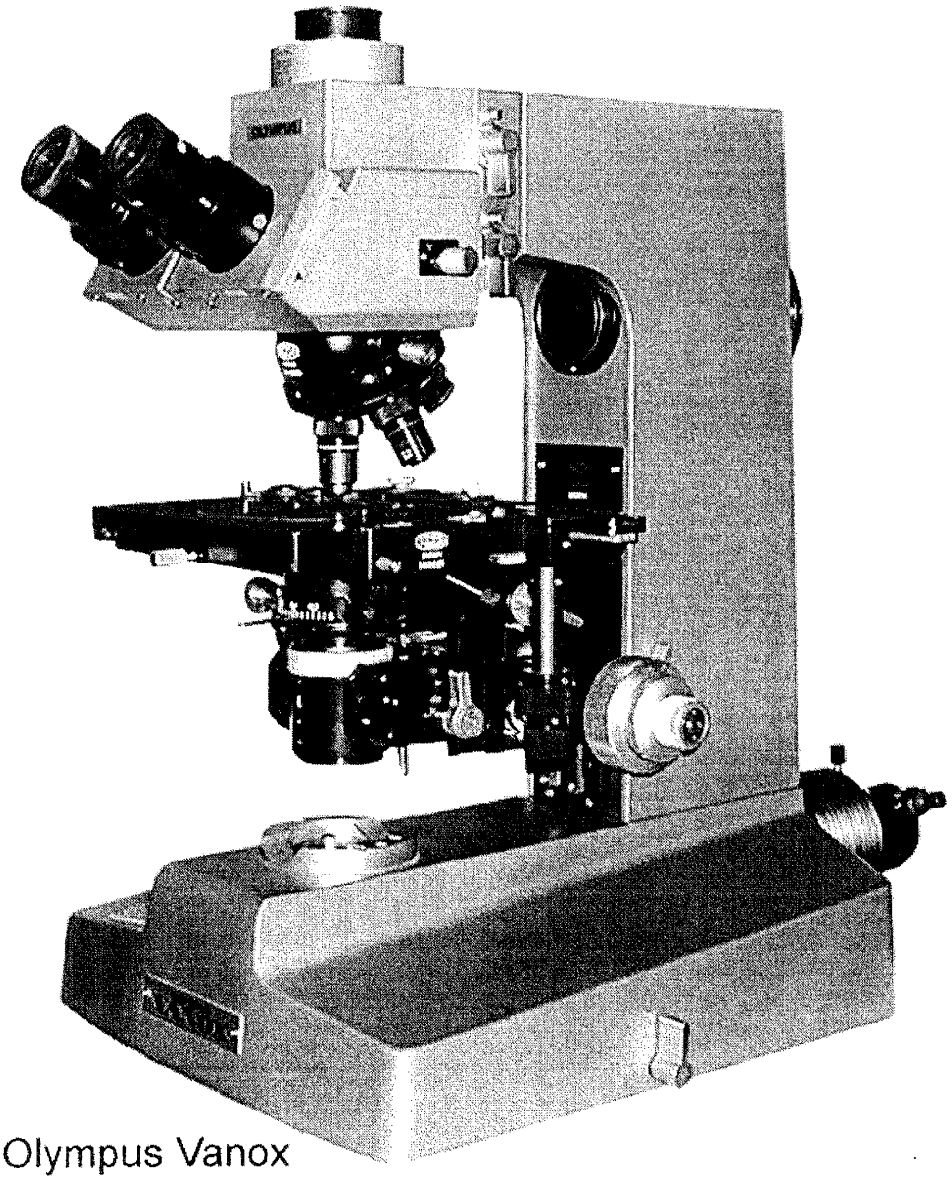

Balloon
theodolite


Aus Jena Spindle Stage Kit

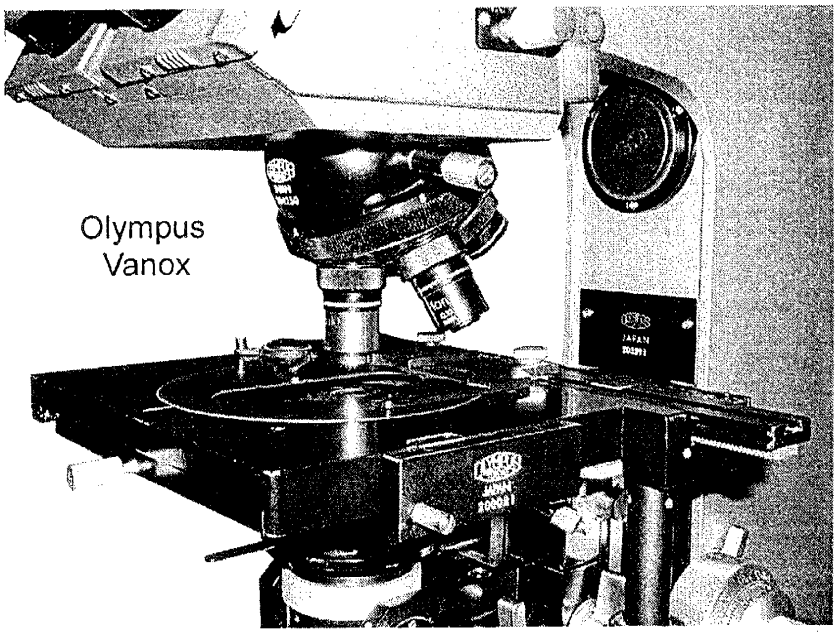

Aus Jena
Spindle Stage



McAllister Microscope with Chain Drive



Olympus Vanox



Olympus
Vanox

LAB NOTES

A Quick Method for Staining Protozoa

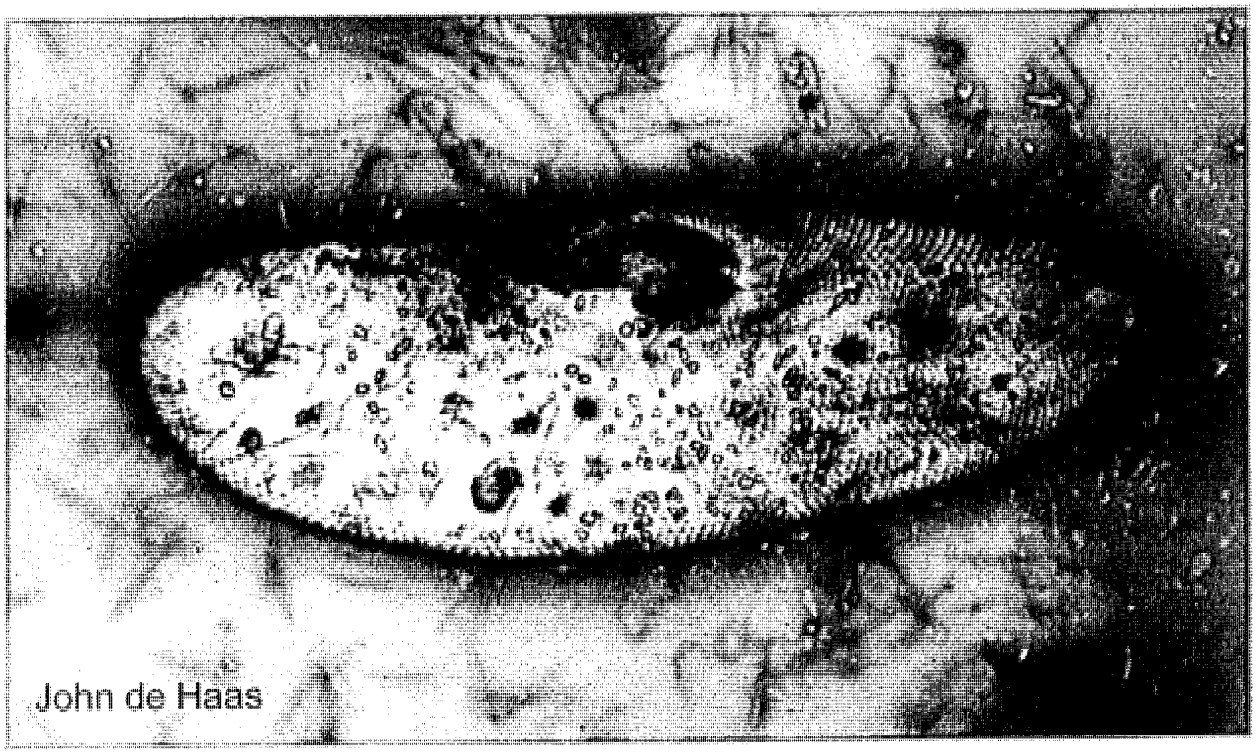
John de Haas

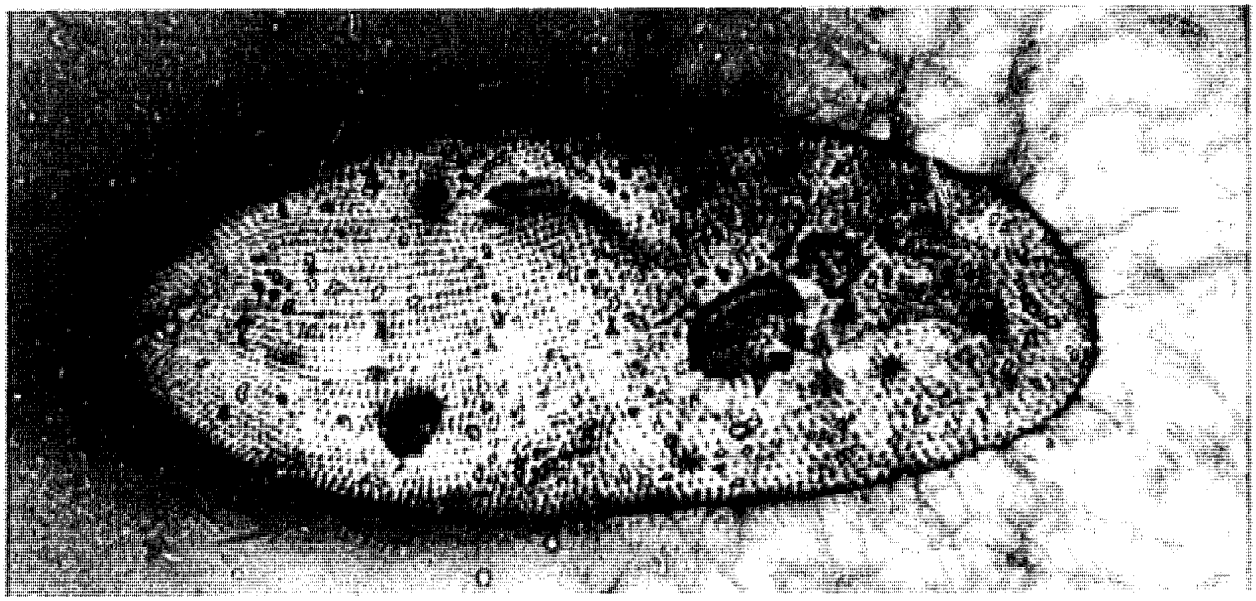
Many years ago, when I was still working in Protozology, I used a technique for making permanent preparations of the larger ciliates. This method is known as Bresslau's method and shows the cuticular structure of the ciliate in great detail. Needed for this work was Opal-Blue, which unfortunately is not available now, so Toloudine-Blue is used instead. It is quite acceptable even though the results are not as uniformly good as with Opal-Blue. The accompanying photos illustrate the results.

Procedure:

Besides a concentrated solution of Toloudine-Blue, a very rich culture of Paramaeciae is needed. Go through the following steps:

1. Take a very clean slide and place a drop of the culture on one end.
2. Next to it place a drop of the dye of about the same size.
3. Gently mix the two drops and make a smear as you would for blood, only much more gentle without much pressure.
4. Let some of the slides air dry and put some others in a simple moist chamber for a few minutes. One never knows which will come out better.
5. After the slides are dry, cover them with mounting medium (Permount or Clearmount will do). Then add a cover slip.





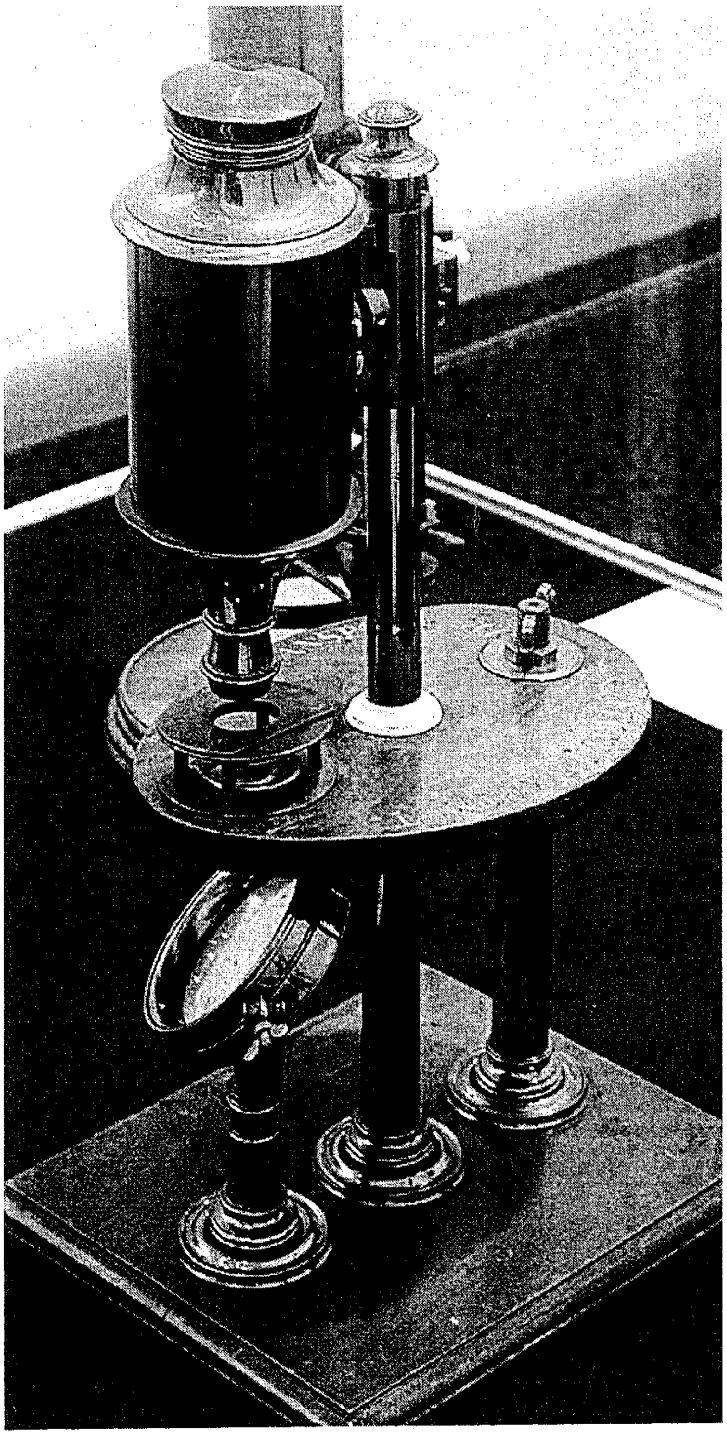
Seeds From Ed Jones' Slide Kit
Continued from page 180

18. Digitalis sp. — Dwarf Red Foxglove
19. Gypsophila paniculata — Baby's Breath Garden Bride
20. Heuchera bressingham
21. Hypericum perferatum — St. John's Wort
22. Jasione sp. — Jasione Blue Light
23. Leptosiphon hybrida — Angel Wings
24. Linaria maroccano — Northern Lights
25. Limonium suwororii — Russian Statice
26. Lobelia erinus — Crystal Palace
27. Lobelia erinus — Lobelia Trailing
28. Lobelia sp. — White Lady
29. Matricaria parthenium — Golden Ball Chrysanthemum
30. Matricaria recutita — German Camomille
31. Mentha piperita — Peppermint (Mint Pepper)
32. Mentha spicata — Spearmint (Garden Mint)
33. Mesembryanthemum crinflorium — Ice Plant
34. Mimulus tigrinus — Monkeyflower
35. Nicotiana alta — Fragrant White
36. Nicotiana sanderae — Sensation Mixed (Tobacco Plant)
37. Nicotiana sylvestris — Only the Lonely
38. Nicotiana tobacum — White Burley Tobacco
39. Oenothera speciosa — Mexican Evening Primrose
40. Origanum heraclitum — Greek Oregano
41. Origanum majorana — Sweet Marjoram
42. Papver nudicaule — Gartford Giants Poppy
43. Papaver orientale — Oriental Scarlet Poppy
44. Papaver rhoeas — Corn poppy or Flanders poppy
45. Papaver rhoeas — Shirley Poppy
46. Papaver somniferum — Single Danish Flag Poppy
47. Petunia hybrida — Petunia Dwarf Bedding Mixed Colors
48. Petunia sp. — Rainbow Mix F-2
49. Polypogon monspeliensis — Beard Grass
50. Portulaca oleracea — Purslane Goldgelber
51. Portulaca grandiflora — Double Mix (Rose Moss)
52. Rucola selvatica (Eruca satium)
53. Rudbecker hirta — Black-eyed Susan
54. Salpiglossis sinuata — Bolero mix
55. Silene armeria — Catchfly
56. Tagacetum vulgare — Tansy
57. Thymus vulgaris — Thyme de Provence
58. Verbena bonariensis
59. Veronica spicata — Speedwell
60. Veronica spicata — Sightseeing Mix

Museum No-Flash / No-Tripod Photographs with the Nikon 990 Digital Camera

Gaylord Moss

During a recent trip to Germany, I had the opportunity to photograph many interesting objects in museums in Berlin, Nuremburg and Munich. Most of these were in glass cases under the restriction that neither flash nor tripod were allowed. Using a Nikon 990 digital camera, I took some photographs that looked acceptable in the LCD monitor on the screen. In the evening in the hotel, however, when I downloaded the photos into my laptop computer, I found that they were all unacceptably fuzzy. At the slow shutter speeds required, I could not hand-hold with sufficient stability. Fortunately, the 900 series Nikon digitals have sufficient capability to meet even these challenging conditions.



Moss photo from Germanisches National Museum Nuremberg

The default effective ASA speed of the sensor system in the 990 is 80. In the manual mode, one can select an effective ASA of as high as 400 which, I presume, the camera accomplishes by increasing the gain of the amplifiers following the optical sensors. (In the later model Nikon 995, the ASA can be increased to 800). Even after setting to ASA 400, the shutter speeds were typically around 1/7 of a second- still beyond my ability to hand hold.

Another Nikon feature came to my rescue. One can set the camera to what is termed the BSS or "Best Shot Select" mode. In this setting, the camera takes a series of shots about a second apart- up to 9 shots if you keep the shutter button depressed. Then the camera automatically analyzes the succession of photos, picks the sharpest image and discards the others. I am told that the internal camera computer makes the judgement with an algorithm which operates on the file size. A larger digital file size indicates more edges and hence a sharper image.

It worked! With the combination of the increased ASA and the Best Shot Selector, the photos downloaded the next evening looked good even on the 15 inch screen of the laptop computer.

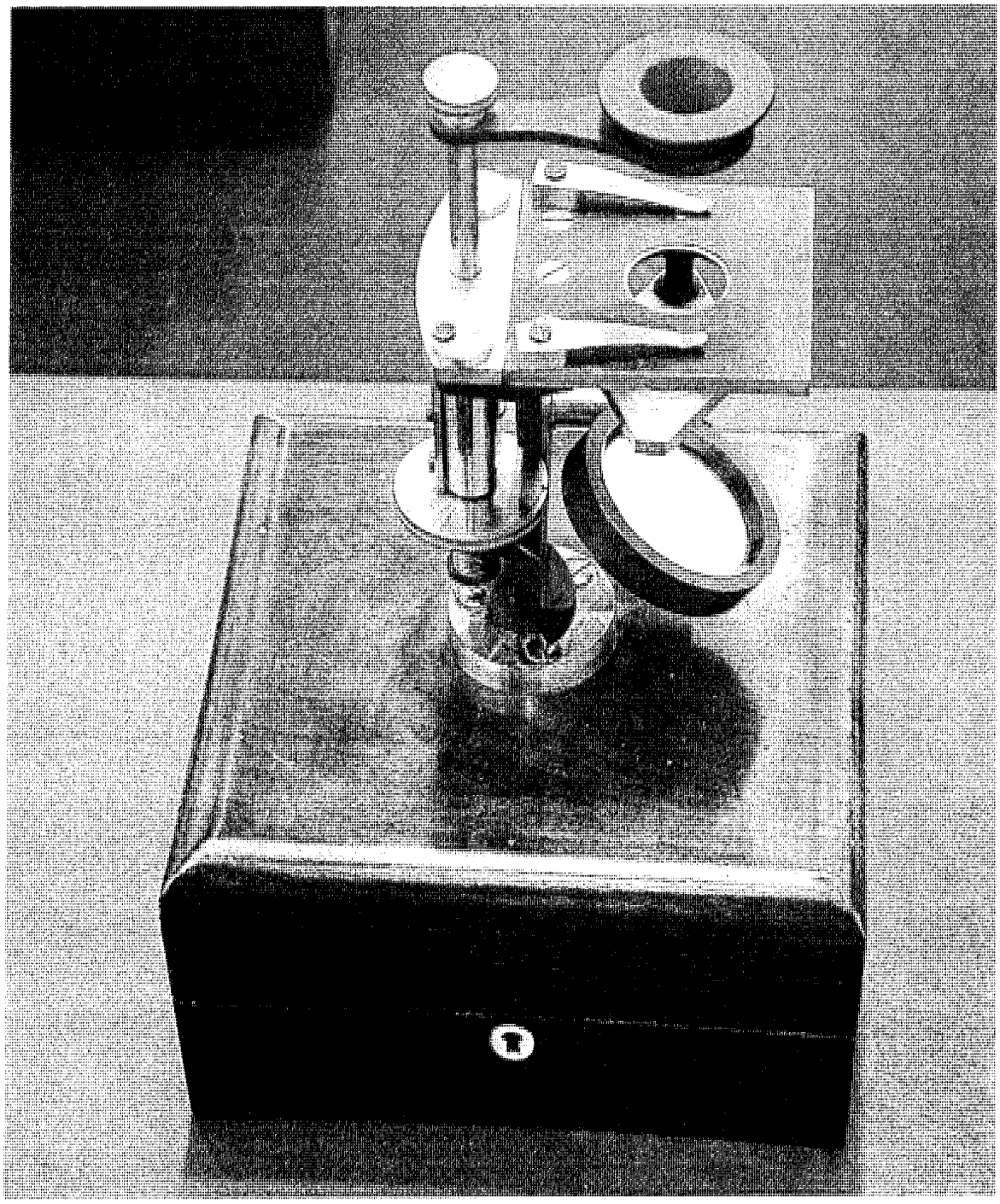
To make it easy to switch from outside photography to the settings needed in the museums, I set one of the three manual modes of the camera to flash-off, BSS-on and ASA of 400. Thus, these settings appeared whenever I set to manual mode. This is another nice feature of the 900 series cameras. One can have essentially four

different cameras each programmed to meet the needs for a particular shooting situation and selected with one switch.

After being forced to figure out this procedure for taking photographs in museum cases, I found that it worked in other places like large rooms where even if flash were allowed, it would not have illuminated the space.

One concern with low light level photographs in a digital camera is with leakage currents in the detectors causing noise in the photos. I did not see any appreciable noise at the light levels that I encountered. Typical shutter speeds were 1/7 of a second at an aperture of f 2.7.

The two photos show the results. Some loss of contrast is attributable to the glass case surfaces through which the photos had to be taken.



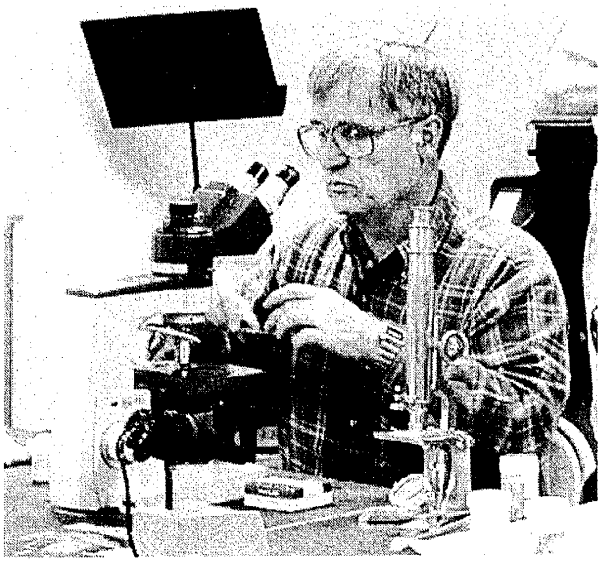
Moss photo from Germanisches National Museum Nuremberg

MSSC Meeting October 17, 2001

7 PM-NewRoads School-
See Map Below

Making a Small Seed Study Slide

Ed JoneS



Anyone who has been fortunate enough to attend one of Ed Jones' previous presentations will not want to miss this event. Ed has prepared individual slide packet materials so that 35 members can make their own slides with 60 varied seeds for study. Ed makes almost superhuman preparations in putting together each kit of many identified objects. He then shares his techniques and varied experience to show us how to duplicate some of his miniature slide making.

Bring to the meeting the tools necessary to create your own seed slide which are:
1. Stereo microscope with 5X to 20X magnification with oblique illumination for opaque objects.
2. Forceps sharp enough to manipulate a single grain of salt.
3. Power cord or extension cord to get power to your illuminator.

Ed Jones will provide a microscope slide with 60 numbered spots, label, sticky tape (mounting media), slide mailer with label and resealable tab, plastic wedges to keep the slide from hitting the top of the slide mailer, list of seeds with scientific and common names and enough seeds for all 60 spots. Ed has enough material for 35 participants.

Arrive early to be assured of one of the kits.

This is a List of the Seeds that Ed Jones has Prepared in each Slide-Making Kit

S

scientific Name	Common Name
1.Achillea fillipenddula	Yarrow
2.Achillea millefolium	Cerise Queen
3.Achillea millefolium	Colorado Mix Yarrow
4.Antirrhinum majus	Snapdragon Magic Carpet
5.Artemesia absinthium	Wormwood
6.Artemesia annua	Sweet Annie
7.Artemesia dracunculoides	Russian Tarragon
8.Artemesia vulgaris	Mugwort
9. Bellis perennis	English Daisy
10. Campanula carpatica	Carpathian Hare-bell
11. Campanula medium	Canterbury Bells
12. Chenopodium ambrosioides	Epazote
13. Chenopodium botrys	Ambrosia
14. Coleus hybridus	Rainbow Mix
15. Digitalis excelsior	Hybrid Foxglove
16. Digitalis purpurea	Foxy Foxglove
17. Digitalis sp.	Foxglove Shirley Hybrid

Continued on page 177