A more detailed description of the apparatus will be published later on.

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Zusammenfassung

Eine Anordnung zur kinematographischen Aufnahme von Spermien von Musterbullen in Dunkelfeldbeleuchtung wird beschrieben. Die Bildfolge von 50 Aufnahmen je Sekunde erfordert eine Lichtquelle von etwa 0,8 kW, welche von einer Spezial-Elektronenblitzlampe geliefert wird.

PRO EXPERIMENTIS

A Method for the Isolation of the Parts of Ciliates[1]

Methods have been devised in this laboratory for isolating quantities of the parts of the infraciliature of *Tetrahymena*, in order that the structure and biochemistry of the ciliary apparatus may be studied. In view of the interest shown by some workers, it has been thought advisable to indicate something of the nature of the method, the kind of results obtainable, and the possibilities of using the method for physiological and morphological analysis of the parts of ciliates.

The method now in use is an adaptation of the method originated by Mazia and Dan[2] and modified by Mazia[3] for the isolation of the mitotic apparatus of dividing sea urchin eggs. In general, it consists of killing the cells by immersion in aqueous-ethanol at sub-zero temperatures, and subsequently transferring them to an aqueous solution of digitonin. The digitonin solution solubilizes much of the cytoplasm of the cell, releasing the nonsolubilized components of the cell so that they are found free in the medium, whereupon they can be collected by differential centrifugation. In the present work, the ciliate *Tetrahymena pyriformis* strain *W* has been grown axenically in 2% proteose-peptone. The cells are concentrated by centrifugation and washed three times with distilled water. The cells are then suspended in 40% ethanol at $-10°$C, and stored at $-10°$ for a few hours. The alcohol suspension is then centrifuged at $-10°$ to collect the cells, the alcohol is decanted, and the sedimented cells are suspended in a 1% aqueous solution of digitonin. The alcohol treatment must never take place above 0°C or the cytoplasm will coagulate and be rendered resistant to the solubilizing action of the digitonin. The solubilization of the cells in digitonin can be followed by observation with phase contrast microscopy.

It has been observed that, when the digitonin suspension is salt-free, all the components of the cell are disrupted and solubilize except the cilia, the kinetosomes, and certain other fibrous structures. The endoplasm, pellicle, and nucleus are dispersed. The kinetosomes forming the membranelles around the mouth are held intact by connecting fibers, and can be isolated *en masse* along with the attached gullet fibers. The somatic kinetosomes (those not in the oral apparatus) do not hold together, except in the anterior polar region where cross-connecting fibers appear to be present. With the dissolution of the pellicle, the cilia are detached from their respective basal granules and float free. Figure 1 is a high-power phase contrast photomicrograph of such a preparation that has been allowed to solubilize under a coverslip so that the kinetosomes retain their natural positions.

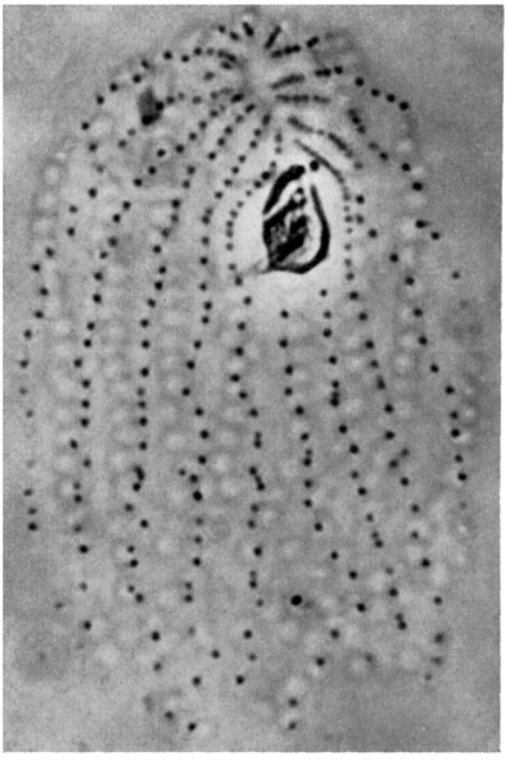

Fig. 1.–Phase contrast photomicrograph of a "solubilized" *Tetrahymena*, showing the kinetosomes and oral apparatus. × 2000.

The structure and arrangement of the infraciliature, especially the associations of kinetosomes and fibers of the oral apparatus, can be seen in such "solubilized" preparations under phase contrast microscopy with a clarity which is approached only by the more tedious methods of silver staining and the "sonicated" preparations used by Metz and coworkers[4]. In addition, these latter methods render the material itself unsuitable for biochemical investigation. Preparations of other protozoa, by the alcohol-digitonin method, have yielded material of further interest. The hypermastigote flagellate *Trichonympha*, obtained from the gut of the termite *Zootermopsis*, has given strikingly clear preparations of isolated anterior ectoplasmic cones with the flagella attached. Pitelka has used this method to produce preparations of opalinid ciliates which have been favorable for electron microscopy and will be described in a later publication.

The dissolution of such structures as the nucleus, pellicle, and mitochondria can be prevented by carefully using a digitonin-KCl solution for solubilization. The nucleus can be prevented from being solubilized if the digitonin solution contains some salt, up to 0·05 *M* KCl.

[1] This investigation was supported by the American Cancer Society, recommended by the Committee on Growth, National Research Council.

[2] D. Mazia and K. Dan, Proc. nat. Acad. Sci. *38*, 826 (1952).

[3] D. Mazia in: *Symposium of the Society for Experimental Biology 9*, 335 (1955).

[4] C. B. Metz, D. R. Pitelka, and J. A. Westfall, Biol. Bull. *104*, 408 (1953). – C. B. Metz and J. A. Westfall, Biol. Bull. *107*, 106 (1954).

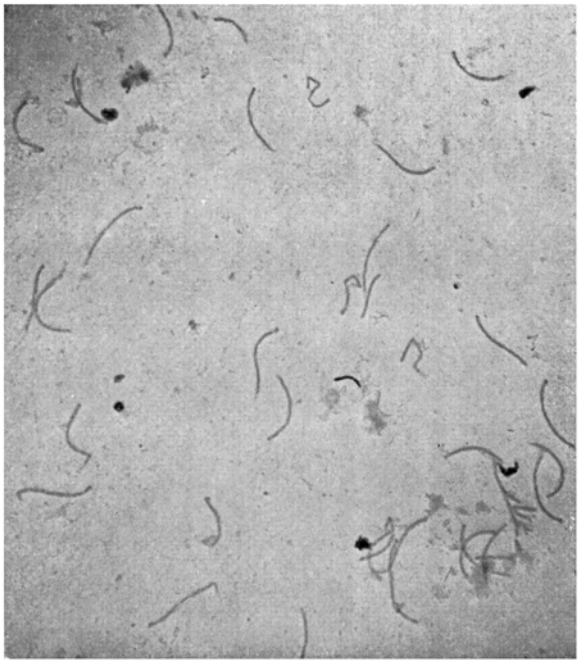

Fig. 2.–A low power electron micrograph of a field of cilia isolated by the alcohol-digitonin method.

Under these conditions, all parts of the cell are less susceptible to digitonin solubilization, but preparations have been obtained which contained many freed nuclei. With higher salt concentrations in the digitonin solution, 0·1 to 0·3 *M* with respect to KCl, all parts of the cell are resistant to digitonin solubilization. At 0·4 *M* KCl, most of the cilia and nuclei disappear, the cytoplasm becomes somewhat clearer, and what appear to be mitochondria located beneath the pellicle in the spaces between kineties, are present. At 0·5 *M* KCl, the cilia, nucleus and most of the cytoplasm dissolve, leaving the refractive pellicle with only faint traces of what look like kinetosomes.

The preparation of mass isolations of the parts of the cell, which have been freed by this procedure of "selective solubilization", is subject only to the usual difficulties attending differential centrifugation. A field of isolated cilia, pictured by low-power electron microscopy, is shown in Figure 2. Kinetosome isolation has been found to be more difficult, largely because of the lack of any criteria for distinguishing them from other small round bodies. Methods are being developed, however, for isolating large quantities of kinetosomes of the oral membranelles since these can be initially isolated as part of the intact oral apparatus. The isolation of nuclei and pellicles, to mention only two of the many fibrous structures found in ciliates, should provide valuable material for biochemical analysis.

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Department of Zoology, University of California, Berkeley, February 10, 1956.

Zusammenfassung

Eine Methode für selektive Solubilisierung der Zellen und verschiedener Organellen von Ciliaten, welche auf einer speziellen Anwendung der Methode MAZIA und DAN zur Gewinnung isolierter Mitoscapparate von Seeigeleiern beruht, wird beschrieben. Die Ciliaten werden bei niedriger Temperatur in 40% Ethanol extrahiert und mit Digitonin behandelt. In salzfreier Digitoninlösung (1% aq) bleiben nur das Wimpernsystem und damit verbundene Strukturen erhalten und können in genügender Menge für chemische Analysen gewonnen werden. Durch Zugabe verschiedener Mengen von KCl ist es möglich, auch Zellkerne, Pellicula, usw. vor der Auflösung zu schützen und durch differentielle Zentrifugierung zu isolieren.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

La question des hétérochromosomes chez les Sauropsidés. Oiseaux.

Par J. M. VAN BRINK et G. A. UBBELS[1], Utrecht

Si le type d'hérédité liée au sexe a été précisé chez les Oiseaux depuis plus de 40 années, la reconnaissance cytologique de la digamétie femelle qu'implique ce type demeure un problème toujours discuté, les travaux parus avant 1949 ayant fait l'objet d'une critique serrée de MATTHEY[2]. Le désaccord qui se manifeste chez les divers auteurs provient en premier lieu de difficultés techniques: les chromosomes sont toujours très nombreux et en majorité de très petite taille (inférieure à 0,5 μ); un tel matériel exige une fixation impeccable en dépit de laquelle l'observation reste très difficile.

L'espèce la plus étudiée est le Poulet, et ce sont les cytologistes japonais (SUZUKI, OGUMA et surtout YAMASHINA[3]) qui ont fait les recherches les plus approfondies sur ce matériel. Ces trois auteurs ont trouvé un nombre de 78 chromosomes chez le mâle, de 77 chez la femelle. La cinquième paire, formée de petits métacentriques, serait représentée par un seul élément dans le sexe femelle.

A en juger d'après les figures publiées, la technique de l'école japonaise (fixations aux liquides de Hermann et de Champy) est infiniment supérieure à toute autre. Malgré cela, les conclusions de YAMASHINA ne peuvent, selon MATTHEY, être acceptées sans réserves: pour cet auteur le décompte objectif de 78 éléments, dont un bon nombre est à la limite de la visibilité, est une chose impossible, ce qui élimine d'emblée tout argument d'ordre numérique en ce qui concerne la question des hétérochromosomes. D'autre part, l'analyse morphologique est très délicate et les possibilités d'erreur nombreuses: la différence entre acrocentriques et métacentriques de taille moyenne ou petite est, dans beaucoup de cas, difficile à apprécier, ce qui rend l'appariement, et par conséquent la découverte d'une paire d'hétérochromosomes très douteux.

NEWCOMER et BRANT[4] ont étudié la spermatogénèse sur des frottis fixés par un mélange complexe à base d'acide propionique et arrivent aux conclusions suivantes: le nombre de chromosomes n'est pas constant

[1] Institut de Génétique de l'Université, Utrecht.

[2] R. MATTHEY, *Les chromosomes des Vertébrés* (F. Rouge, Lausanne 1949).

[3] Y. YAMASHINA, Cytologia *13*, 270 (1944).

[4] E. H. NEWCOMER et J. W. A. BRANT, J. Heredity *45*, 79 (1954).