PROPERTIES OF DNA ASSOCIATED WITH RAFFINOSE-ISOLATED PELLICLES OF PARAMECIUM AURELIA

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SUMMARY

Pellicular fractions, rich in basal bodies, have been obtained from Paramecium aurelia after the animals were homogenized in the presence of buffered raffinose. DNA was extracted from these fractions. This DNA was examined with respect to density and to its susceptibility to digestion by DNase while still associated with pellicles, and was found to be of two kinds: (a) Most resembles nuclear DNA in density, is completely removed from pellicles by pancreatic DNase and forms a broad band when centrifuged to equilibrium in cesium chloride. This DNA may be of nuclear origin, although a pellicular source cannot be ruled out. (b) Several minor species of DNA with densities of 1.698, 1.703-1.704 and 1.717-1.718 g/cm³ (1.698 x 10³, 1.703-1.704 x 10³ and 1.717-1.718 x 10³ kg m⁻³, respectively) are also present. These species were not digested when pellicles were treated with DNase. None of these DNA species occurs consistently in all preparations of pellicular DNA and the results indicate that they are probably attributable to bacteria present during culture of the animals in non-axenic medium.

To test the ability of pellicles to become associated with exogenous DNA during isolation, DNA of Escherichia coli, labelled with [³H]thymidine, was added to whole cell homogenate before isolation of pellicles. Comparison of the specific activities of DNA extracted from the homogenate with that from isolated pellicles provided evidence that exogenous E. coli DNA can bind to pellicles.

These observations suggest that pellicles or basal bodies may become contaminated with nuclear DNA during isolation. This would create a serious problem, which must be rigorously dealt with in experiments designed to demonstrate the existence of basal-body DNA in isolated pellicles by cytochemical, biochemical or autoradiographic methods. The results indicate that it has not been established beyond reasonable doubt that basal bodies or centrioles contain DNA. In addition, the results suggest that further verification of existing evidence for basal-body DNA may require further sophistication of existing analyses or a novel approach, involving different biological material or new techniques.

INTRODUCTION

Within the past few years it has been acknowledged that certain extranuclear organelles (mitochondria and chloroplasts) of eucaryotic cells contain DNA (Kirk, 1966; Granick & Gibor, 1967; Swift, 1965). Studies by Randall & Disbrey (1965), using staining with acridine orange and autoradiography on isolated pellicles of Tetrahymena, suggest that ciliary basal bodies (kinetosomes) also may contain DNA. This view is supported by similar studies on Paramecium aurelia by Smith-Sonneborn &
Plaut (1967), and by an autoradiographic study on *Opalina ranarum* by Sukhanova & Nilova (1965). Furthermore, Seaman (1959) and Hoffman (1965) report that small amounts of DNA can be extracted from isolated basal body preparations of *Tetrahymena pyriformis*. In none of these studies was the DNA characterized physically to eliminate the nucleus as a possible source of contaminating DNA.

Techniques have recently been developed (Hufnagel, 1969) for obtaining basal body-rich pellicular fractions of *Paramecium aurelia*. No satisfactory method has yet been found for separating basal bodies of paramecium from associated membranous and fibrillar structures. Therefore, whole pellicles were used in initial studies of pellicle-associated DNA, in the hope that such experiments would eventually lead to the characterization of DNA specifically associated with basal bodies.

DNA was extracted from isolated pellicles of stock CD and 51. Utilizing a variety of physical and chemical techniques, the properties of the extracted DNA have been examined. No evidence was obtained that any of the DNA associated with isolated pellicles is of basal body origin. Furthermore, the results of these studies support the belief that the existence of basal body DNA has not been clearly established. Some of these observations have been described briefly in previous publications (Hufnagel, 1965, 1966).

**MATERIALS AND METHODS**

*Culturing and harvesting*

The animals studied were stocks CD (a syngen 1 derivative isogenic with stock 90 except for the presence in homozygous condition of the recessive mutant alleles, cl, dp, mt, d80 and g60 (Kimball & Gaither (1955), kindly supplied by Dr Kimball) and 51 (sensitive) of *Paramecium aurelia*. The animals were grown in 5-gal (0.02273 m³) jugs on an infusion of buffered Scottish grass inoculated with *Aerobacter aerogenes* and were harvested with the aid of a modified cream separator as described by Preer & Preer (1959). Prior to isolation of pellicles, animals were usually stored 1-4 days in exhausted culture fluid, at 15 °C. Thus, pellicular DNA was obtained from non-dividing animals.

*Isolation of pellicles*

Details of the isolation procedure have been given elsewhere (Hufnagel, 1969). In general, concentrated animals were resuspended in 9-10 volumes of ice-cold phosphate-buffered 0.2 M raffinose (pH 6.8) and homogenized with a Potter–Elvehjem glass and teflon homogenizer. Pellicles were then obtained by at least 6 sequential low-speed centrifugations (480 g, 5 min), with resuspension of the pelleted pellicles in raffinose medium or phosphate buffer at each step. Figure 3 is a phase-contrast micrograph of a typical pellicle isolate prepared in this fashion. Pellicle fractions utilized for extraction of DNA were always visibly quite free of particulate contamination.

*Isolation of mitochondria*

In two cases, after pellicles were removed from an homogenate by centrifugation, mitochondria were isolated from the remaining supernatant. The mitochondria were collected by centrifugation of the supernatant at 12,100 g for 10 min. The supernatant layer was poured off and uppermost layers, containing primarily cilia and trichocysts, were removed by pipette. The mitochondrial fraction was then washed 3 times with raffinose medium, by repeated centrifugation at 12,100 g, until phase-contrast microscopy indicated that visible contamination by non-mitochondrial particles was minimum.
Extraction of DNA

The extraction method is a modification of that described by Suyama & Preer (1965). Pellicles from 3 to 6 ml of packed cells were suspended in about 0.5 ml total volume of phosphate buffer (0.01 M, pH 6.8) or buffered raffinose, and diluted to 20 ml with saline EDTA (0.15 M NaCl, adjusted to pH 8.0 with concentrated NaOH). To lyse pellicles, 0.2 ml of 25% sodium dodecyl sulphate (SDS; contains 95% sodium lauryl sulphate) was added with stirring. The mixture was extracted with an equal volume of phenol (distilled crystalline carbolic acid (Merck), 10 ml; saline EDTA, 2 ml; saturated NaOH, 10 drops), by shaking gently in an ice-water bath for 20 min. After gentle centrifugation to separate phases, the aqueous phase was collected, exhaustively dialysed against BPES (0.17 M NaCl; 0.005 M NaH₂PO₄; 0.002 M Na₂HPO₄; 0.001 M EDTA Na₂ (Crothers, 1964)), and concentrated to 3 to 5 ml by evaporation through cellulose dialyser tubing at room temperature. After further dialysis against BPES, the DNA-containing solution was centrifuged at 21700 g for 10 min to remove insoluble material.

DNA was extracted from 0.5 ml of packed whole cells and from a mitochondrial fraction from 6 to 10 ml of cells using similar methods. Extracted DNA was stored at 4 °C for periods up to 12 months, or until used for measurements.

Determination of concentration

Ultraviolet spectra were obtained on all nucleic acid solutions. Because some non-nucleic acid material was present, O.D.₂₄₀/O.D.₂₆₀ ratios ranged from 0.46 to 0.52 and O.D.₂₆₀/O.D.₅₄₀ ratios varied from 0.57 to 0.81. A rough estimate of DNA concentration was obtained from the kinetics of digestion by DNase; the Beckman DU and Gilford attachment were used to record increase in hyperchromicity at 260 nm. For more accurate measurements, colour reaction with indole (Keck, 1936) was used.

Density gradient centrifugation in cesium chloride

Optical grade CsCl (The Harshaw Chemical Co.) was used. Analytical density-gradient centrifugation was carried out according to the method of Schildkraut, Marmur & Doty (1962) in a Beckman model E analytical ultracentrifuge equipped with u.v. optics. DNA from the bacteria Rhodopseudomonas palustris and Rhodospirillum rubrum (both having a density at 20 °C of 1.725 g cm⁻³ (1.725 × 10³ kg m⁻³); Suyama & Gibson, 1966) was used as standard.

The samples were centrifuged at 44770 rev min⁻¹ at 20 °C for 18 to 22 h. Ultraviolet absorption photographs were taken with Kodak commercial film and tracings of them were made with a Joyce, Loebl & Co. Ltd. double-beam recording microdensitometer, model E12MKIII. Density of u.v.-absorbing bands was calculated by the method of Schildkraut et al. (1962).

Density-gradient centrifugation was also carried out on heat- and DNase-denatured DNA. For heat denaturation, 0.4 ml of a solution of DNA in BPES was heated in a boiling water bath for 15 min. It was then mixed rapidly with 0.8 ml cold (4 °C) BPES in an ice-water bath, to preserve the denatured state. Digestion by DNase was performed in BPES, with the addition of 0.1 M MgCl₂ to give a final concentration of 0.01 M and pancreatic DNase in BPES (5 mg/ml) to give a final concentration of 50 μg/ml. The course of digestion by DNase was monitored spectrophotometrically at 260 nm.

Isolation of [³H]thymidine-labelled DNA from E. coli T'-A'-U'

E. coli stock cultures were grown in test tubes on Vogel’s medium (50X: 10 g MgSO₄·7H₂O; 100 g citric acid·H₂O; 500 g K₂HPO₄; 175 g Na₂HPO₄·4H₂O; dissolve successively in 670 ml H₂O and add H₂O to 1 l, then add 1 ml CHCl₃ containing 20 ml/l. 25% glucose, 20 mg/l. uracil, 20 mg/l. arginine and 20 mg/l. thymidine. To obtain radioactively labelled cells, 1 ml of a stock culture was inoculated into a 2-l. flask containing 1 l of a medium similar to that described above but containing only 2 mg/l. thymidine. The inoculated medium was incubated with constant shaking at 37 °C for 15 h. Cells were collected by centrifugation at 16300 g for 20 min.

Packed cells (1 to 2 g wet weight) were diluted about 1:500 with cold saline EDTA and lysed
by the addition of 25 % SDS to a concentration of 0.25 %. The mixture was stirred gently for several minutes in a 60 °C water bath, 500 ml cold alkaline phenol was added and the mixture stirred at ice-water temperature for about 20 min. The aqueous phase was collected and nucleic acids precipitated by addition of 2 volumes of cold (4 °C) 95 % ethanol. DNA was collected on a glass rod, redissolved in BPES and dialysed against BPES. The DNA solution was then centrifuged at 21 700 g for 10 min. In 2 separate experiments, total amounts of DNA obtained in this way were 2400 μg and 2700 μg, measured colorimetrically.

**Scintillation counting**

Radioactivity of [3H]labelled DNA was measured with a Nuclear Chicago 720 series liquid scintillation counter. Five to 100 lambda aliquots of each DNA solution were dropped on to 22-mm membrane filter disks (Bac-T-Flex), the filters were dried under an infra-red lamp, placed in glass vials and 10 ml of scintillation fluid (2,5-diphenyl-oxizole, 60 g; 1,4-bis-2(5)-phenyloxozolyl benzene, 150 mg; toluene, 1500 ml) were added to each vial. A blank disk was used for background. Measurements were averages of 3 or 4 samples.

During isotope studies, to test whether 3H-label was in *E. coli* DNA, aliquots (0.5 ml) of DNA stock solutions in BPES were treated at room temperature for 0.5 h with 0.05 ml of DNase (5 mg/ml in BPES) in the presence of 0.01 M MgCl₂. Controls without DNase and MgCl₂ were run simultaneously. After digestion, 0.1-ml aliquots were spotted on to separate 23-mm Whatman No. 1 filters. These were immediately placed in a beaker containing cold (ice-water temperature) 10 % trichloroacetic acid (TCA) for 10-15 min. Several unspotted blanks were run simultaneously. After 3 washes in cold TCA, TCA was removed by decanting and replaced with 3:1 ethanol:ethyl ether. After 10 min at room temperature, this was replaced with pure ether for 5 min. The filters were then spread on aluminium to dry, and counted as described above.

**RESULTS**

**Properties of DNA associated with pellicles**

In nine separate extractions pellicular fractions (see Fig. 3) yielded from 1.6 to 20 μg of DNA per cc of packed cells used to isolated pellicles. These yields were obtained when pellicles were washed 5 or 6 times before extraction of DNA. Pellicles washed 8 to 12 times yielded only slightly less DNA. Total extractable pellicular DNA represents of the order of 0.1 % of the total cellular DNA (assuming 2 mg of DNA per cc of packed cells—see part four of Results).

Utilizing analytical ultracentrifugation in CsCl density gradients, DNA extracted from whole animals and pellicles of stock CD were compared (Fig. 1). Pellicular DNA formed a single broad band corresponding to a density of 1.686-1.688 × 10³ kg m⁻³. This band was found to be sensitive to DNase and was denatured by heat, with a shift in density of -0.018 × 10³ kg m⁻³. Densities of whole cell and pellicular DNA were found to correspond within 0.001 x 10³ kg m⁻³ (whole cell: 1.687 × 10³).

The density of pellicular DNA of stock 51 was also examined, because techniques employed in our laboratory showed that the density of the major DNA component of whole cells of stock 51 (1.691 × 10³ kg m⁻³) is significantly different from that of stock CD (S. Pollack, personal communication). (However, the major species of DNA of stock 51 has been reported by Smith-Sonneborn, Green & Marmur (1963) to have a density of 1.689 × 10³ kg m⁻³.) The density of pellicular DNA from stock 51 (1.691 × 10³ kg m⁻³) was found to be identical to that of whole cell DNA from stock 51. In other words, the DNA extracted from pellicles of each stock resembled in density the major DNA component (presumably of nuclear origin) present in whole cells of the
corresponding stock. These observations suggest that a portion of the DNA associated with pellicles may be of nuclear origin, but it is also possible that there exists a specific pellicular DNA resembling nuclear DNA in density.

Fig. 1. Densitometer tracings of ultraviolet absorbing peaks appearing after equilibrium centrifugation of nuclear and pellicular DNAs of stock CD and 51 in the presence of CsCl. The reference DNA, *Rhodopseudomonas palustris*, was previously standardized against deuterium-labelled *B. subtilis* DNA (lowest tracing).

**Studies utilizing *E. coli* DNA labelled with [³H]thymidine**

To examine whether exogenous DNA, such as nuclear DNA, can in fact bind to pellicles, in three separate experiments, *E. coli* DNA labelled with [³H]thymidine was added to an homogenate of whole cells from which pellicles were to be isolated. In each case, an aliquot of the homogenate, representing about 0·5 ml of packed cells, was then removed and DNA was extracted from it. The remainder of the homogenate was utilized for isolation of pellicles by the usual procedure. DNA was extracted from the pellicular fractions. In two experiments, mitochondria were also isolated from the same homogenate. In one experiment, the pellicles were resuspended in 0·01 M phosphate buffer (pH 6·8), divided into two equal aliquots, and one aliquot was treated with pancreatic DNase (50 μg/ml final concentration) for 0·5 h in the presence of 0·005 M MgCl₂, in an ice-water bath. The specific activity for each sample of DNA was determined.

To check that most of the counts in these experiments represent labelled DNA, samples of DNA isolated from mitochondria and whole cells were treated with pancreatic DNase. DNase-treated and control samples were spotted on to filters and the
radioactivity adhering to the filters was measured by scintillation counter. In the one experiment in which DNase was used (experiment 3, to be described presently), at least 90% of the counts were digestible by DNase.

Results of the three experiments are given in Table 1. DNA isolated from pellicles was found consistently to have a slightly greater specific activity (980 CPM/µg of DNA in Expt. 3) than DNA from unfractionated homogenate (688 CPM/µg of DNA). This increase in specific activity can be explained if DNA of *E. coli* preferentially binds to pellicles.

Table 1. Results of isotope dilution experiments*

<table>
<thead>
<tr>
<th>Specific Activity</th>
<th>WC+</th>
<th>MIT+</th>
<th>PELL+</th>
<th>PELL + DNase</th>
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<tr>
<td>Relative Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>MIT</td>
<td>1.0</td>
<td>0.36</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>PELL</td>
<td>1.38</td>
<td>1.29</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>PELL + DNase</td>
<td></td>
<td></td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>DNA yield (g^3H/kg net)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>4</td>
<td>27</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>MIT</td>
<td>0.38</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PELL</td>
<td></td>
<td></td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>PELL + DNase</td>
<td></td>
<td></td>
<td>1.3</td>
<td></td>
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</table>

* Results given in CPM/µg DNA. WC, DNA from whole cell homogenate; MIT, DNA from mitochondrial fraction; PELL, DNA from pellicular fraction.

On the other hand, DNA isolated from mitochondria was found to have a specific activity which is lower than the activity of DNA from whole cells (250 CPM/µg in Expt. 3). Decrease in specific activity can be explained if mitochondria contain their own specific DNA. This has been shown to be true of the mitochondria of *Paramecium* (Suyama & Preer, 1965).

Therefore, experiments with exogenous DNA might be used to reveal the presence of a specific pellicular DNA. However, if *E. coli* DNA binds to pellicles, by increasing the specific activity of pellicular DNA it would obscure the presence of a specific pellicular DNA. On the other hand, if a specific pellicular DNA does exist and its association with the pellicle survives the isolation procedure, this DNA may be resistant to exogenously added DNase, while non-specifically bound DNA (including *E. coli* DNA) should be preferentially digested. To examine this possibility a portion of the pellicles in isotope Expt. 3 was treated with DNase.

As shown in Table 1, after pellicles were treated with DNase the specific activity of pellicular DNA dropped to a value of 2 of that for DNA from untreated, unfractionated homogenate. While this reduction could be explained in a number of ways, it might indicate that a pellicle-specific DNA is present.
DNA found with pellicles of Paramecium

Treatment of pellicles with DNase, prior to extraction of DNA

To examine further the in situ susceptibility of pellicle-associated DNA to digestion by DNase, the DNA from DNase-treated and untreated pellicles was compared by centrifugation in a CsCl density gradient. In each experiment the DNA added to the ultracentrifuged cells represented approximately equivalent amounts of DNase-treated and -untreated pellicles. Results of one such study are given in Fig. 2. Essentially the same results were obtained in other studies.

![Figure 2](image)

Fig. 2. Densitometer tracings of ultraviolet absorbing peaks appearing during equilibrium centrifugation of DNA extracted from control and DNase-treated pellicles. Note that the majority of DNA is removed from pellicles by DNase. DNase-resistant peaks seen here do not occur consistently in all preparations.

As evident in Fig. 2, the majority of pellicular DNA, having a mean density similar to that of DNA from whole cells (1.686–1.688 × 10^3 kg m⁻³), is completely removed from pellicles with DNase. In these studies several other species of DNA (densities of 1.698, 1.703–1.704, and 1.717–1.718 × 10^3 kg m⁻³) were also encountered; these were observed to be virtually unaffected by the DNase treatment. Presence of these species of DNA accounts for the decrease in specific activity of pellicular DNA when pellicles are treated with DNase. Several lines of evidence are consistent with the interpretation that these other DNA peaks originate from bacteria present in cultures. (i) Satellite bands do not occur consistently from preparation to preparation. Different species occur in different preparations and in some preparations of pellicular DNA no satellite species were observed. (ii) A number of bacterial forms were known to be present in
cultures of *Paramecium* utilized in this study and in pellicular fractions. These forms include ones having DNAs with densities of 1.698 (see below), 1.717 (*A. aerogenes*, used as a food organism) and 1.696 and/or 1.703 x 10^3 kg m^-3 (*Bacillus* species: Schildkraut et al. (1962)). (iii) Satellite DNA species are resistant to digestion when pellets are treated with DNase.

Attempts were made, by counting and plating methods, to estimate the number of bacteria contaminating pellicular samples, but because the bacteria tended to clump with one another or with pellicles, a valid estimate was not obtained. In one study isolated pellets were plated out on Penassay medium, the bacterial colonies which formed were scraped up and DNA was isolated from them. Using centrifugation in a CsCl density gradient, the density of this DNA was found to be 1.698 x 10^3 kg m^-3.

**DNA of whole cells**

The data obtained in the three isotope experiments were also used to calculate the amount of DNA present in whole paramecia. The calculation was based on the ratio of the specific activity of the added *E. coli* DNA to that of the whole cellular DNA.

Table 2. The amount of DNA present in whole cells of *Paramecium aurelia*, stock CD

| Expt. | Spec. act. of *E. coli* DNA | Spec. act. of whole cell DNA | *E. coli* DNA added, µg | Calc. DNA | Extr. DNA
<table>
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<tbody>
<tr>
<td>1</td>
<td>1.09 x 10^4</td>
<td>1.6 x 10^3</td>
<td>228</td>
<td>2.584</td>
<td>1.340</td>
</tr>
<tr>
<td>2</td>
<td>1.09 x 10^4</td>
<td>2.75 x 10^3</td>
<td>288</td>
<td>1.524</td>
<td>1.114</td>
</tr>
<tr>
<td>3</td>
<td>1.6 x 10^4</td>
<td>6.88 x 10^3</td>
<td>345</td>
<td>0.804</td>
<td>0.825</td>
</tr>
</tbody>
</table>

* (CPM/µg DNA). † Calculated from ratio of specific activity of whole cell DNA to spec. act. of *E. coli* DNA; mg/ml cells. ‡ DNA actually extracted as determined by Keck's method (1956); mg/ml cells.

Table 2 gives results of these calculations and the results of colorimetric measurement of extractable DNA. It can be seen that in the first two experiments there was at least 1.5 times more DNA than is extracted by the procedure used. Some unextracted DNA probably remains trapped in phenol-precipitated protein. The failure to confirm this finding in the third experiment remains unexplained.

**DISCUSSION**

Evidence has been presented that isolated pellicles of *Paramecium aurelia* contain small amounts of DNA. Yields were found to vary by a factor of 100. This variation suggests that most of the extracted DNA is not localized in a consistently occurring component of the pellicles; the experiments to be described below support this view. However, assuming 1/10 dilution at each wash, it is estimated that pellicles contain 1000 to 10000 times more DNA than would result from simple dilution of nuclear DNA. These considerations suggest that the majority of DNA found with pellicles
is not of pellicular origin, but that pellicles have some affinity for exogenous DNA. Evidence derived from studies with [3H]thymidine-labelled DNA of *E. coli* supports this interpretation. In addition, the majority of DNA associated with pellicles was found to resemble in density nuclear DNA, even when pellicular DNA of stocks having nuclear DNA differing in density were examined.

In some CsCl density gradients additional satellite bands were observed (densities: 1.698, 1.703–1.704 and 1.717–1.718 × 10^3 kg m^{-3}). None of these species occurs consistently in all preparations of DNA from pellicles and they are probably attributable to bacteria present in the culture medium.

Attention is drawn to the heterogeneity in CsCl density gradients of the major species of DNA isolated from pellicular fractions. Heterogeneity may indicate molecules of small size, since *Paramecium* may have very active nuclease, or it may reflect a population of DNA molecules of differing densities. (The presence of active nuclease in paramecium homogenates seems likely for two reasons: (i) food vacuoles, which contain considerable deoxyribonuclease activity (Muller, 1962), are broken open upon homogenization; (ii) nuclear DNA can be collected on a glass rod only if cell lysis and phenol extraction are carried out very quickly (less than 2 min) and in the cold. Surprisingly little information is available in the literature concerning this very important possibility. A test for deoxyribonuclease activity in homogenates of *Tetrahymanella* by Mita & Scherbaum (1965) was negative but sensitivity of the method was extremely low and the nature of the test leaves open the possibility of enzyme inactivation.) It is therefore possible that this major band may harbour a non-nuclear DNA existing in small quantities. If a specific pellicular DNA is thus hidden, it is estimated on the basis of the data on centrifugation that it would occur in quantities of not more than 10^{-16} g per basal body (assuming 10^3 basal bodies per animal and 100% yield of pellicles from whole cells). However, our data show that, unlike mitochondrial DNA (Luck & Reich, 1964), such pellicle-specific DNA, if it exists, would have to be susceptible *in situ* to DNase attack under the conditions used to isolate pellicles.

Of course, there may be pellicle DNA which is resistant to DNase digestion but insufficient in quantity to be detectable in these experiments. In one of the DNase experiments (see Table 1), the total DNA obtained from DNase-treated pellicles was 1.3 × 10^{-16} g/basal body. In this experiment a satellite peak representing no more than 30 to 50% of the total DNA isolated from DNase-treated pellicles was detectable. Let us assume that a peak of half this size or smaller would not be visible; undetectable DNA could amount to as much as 25% of the total DNA. If these assumptions are made, it can be estimated from the data that undetectable DNA could have amounted to as much as 3.2 × 10^{-17} g/basal body.

Another possibility which must be considered is that there is a pellicle-specific DNA which is, however, lost from the pellicles during their isolation. Electron microscopy (Hufnagel, 1969) indicates that pellicles isolated in the fashion described above retain a nearly entire complement of basal bodies (the presumed site of a pellicle-specific DNA). However, it is difficult to determine, from electron micrographs, whether possible DNA-containing portions of the basal bodies have been preserved. DNA could be lost from pellicles through nuclease activity (see evidence cited above).
Loss of DNA from the pellicles during their isolation has not been discounted in the present experiments.

In addition, the present studies do not deal with the possibility that the methods used to isolate DNA may not extract DNA from basal bodies. However, this does not appear likely, since SDS, at the concentrations used, dissolves basal bodies (Hufnagel, 1969).

Do basal bodies contain DNA? One cannot dismiss evidence for DNA in basal bodies obtained in studies on ciliates stained with acridine orange and labelled with [3H]thymidine (Randall & Disbrey, 1965; Smith-Sonneborn & Plaut, 1967; Sukhanova & Nilova, 1965). In studies on ethanol-isolated 'ghosts' of *Tetrahymena* (Randall & Disbrey, 1965) and *Paramecium* (Smith-Sonneborn & Plaut, 1967) basal bodies stained with acridine orange exhibited green fluorescence. This was interpreted as evidence for the presence of double-stranded DNA (Bradley, 1966; MacInnes & Uretz, 1966). In both studies, maximum staining was observed during specific stages of the cellular division cycle, suggesting that 'basal body DNA' undergoes changes in quantity or physical state.

In autoradiographic studies of the incorporation of [3H]thymidine into isolated pellicles (Randall & Disbrey, 1965; Smith-Sonneborn & Plaut, 1967) further evidence for basal body DNA was obtained. Light microscopy showed that patterns of labelling closely parallel the arrangement of basal bodies in the pellicle.

However, observations at the electron-microscopic level (Pyne, 1968) yielded negative results. Pyne, in studies on *Tetrahymena*, compared the amount of label over mitochondria and basal bodies. He concludes that a basal body must contain a great deal less DNA than a mitochondrion (3-5 x 10^{-16} g/mitochondrion (Suyama & Preer, 1965)). One hundred times less DNA could code for about 1000 amino acids—not enough to form a respectable protein.

Several obvious sources of error must be dealt with in cytochemical and autoradiographic studies. First, if isolated pellicles are used the possibility of contamination from nuclear or mitochondrial DNA must be ruled out. Secondly, it must be shown that staining or labelling is due to DNA rather than some other substance. This is a particularly important consideration with acridine orange, since green staining can be obtained with double-stranded RNA (Bradley, 1966) or proteins and carbohydrates (see Kasten, 1967, for review of this subject). In addition, thymidine can be expected to give up its label to other compounds during long-term incubation. Thirdly, localized labelling or staining must clearly be shown not to be due to DNA in mitochondria still attached to isolated pellicles.

The present studies were carried out because it was felt that unequivocal evidence for basal body DNA would only be obtained through the direct approach of chemical and physical characterization of DNA isolated from basal bodies. The studies reported herein provide a partial characterization of DNA extracted from isolated pellicular fractions of *Paramecium* when such pellicles are obtained by homogenization of animals in raffinose medium. When this DNA was examined by chemical and physical methods found useful for characterizing DNA of other organelles, no evidence for pellicle-specific DNA was obtained. On the contrary, it seems likely that the DNA
present in such preparations originates from nuclei or bacteria. If these conclusions are
correct, it is estimated that the largest amount of DNA which might be present and
not be detected by these experiments is not more than $3.2 \times 10^{-17}$ g/kinetosome.

This study and that of Pyne (1968) increase the likelihood that the basal body does
not contain DNA functional in the usual sense in coding for proteins or that the
amount of DNA present is far smaller than occurs in other cytoplasmic organelles.
If the latter is true, visualization of this DNA by chemical or physical means may
require new approaches not necessary for satellite DNA species heretofore studied.
Since the only evidence for basal body DNA comes from ethanol-isolated pellicles,
some new approaches are being conducted on pellicles of *Tetrahymena* isolated in the
presence of ethanol (Hufnagel, 1968).

If basal bodies do not contain DNA, their replicative functions can probably be
explained in terms of an RNA code or in terms of protein-protein interactions, as
pointed out by Pyne. In addition, the basal body may function in generating other
organelles or in self-replication, by serving as a site for specialized protein synthesis,
using messengers synthesized in the nucleus. Some evidence does exist, in the case of
*Paramecium* (Dippell, 1968, and personal communication), for the presence of RNA
in basal bodies. It may be profitable, therefore, to characterize the RNA and proteins
of basal body fractions, using biochemical and biophysical methods, and to ask whether
basal bodies can incorporate amino acids into proteins.

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Fig. 3. Phase-contrast micrograph of a preparation of raffinose-isolated pellicles similar to preparations used for extraction of DNA. × 800.