SOME OBSERVATIONS ON THE ISOLATION OF MITOCHONDRIA FROM TETRAHYMENA PYRIFORMIS GL[1]

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Despite the numerous biochemical studies which have been performed on the ciliate Tetrahymena pyriformis, relatively few workers have attempted to study the chemistry of cell-free particulate systems derived from this organism. In view of the increased interest currently being shown this species as a result of the introduction of synchronous division (Scherbaum and Zeuthen, 1954) this laboratory has attempted to define a workable fractionation scheme for obtaining such particles. A portion of this work concerning the ribosomal complement and some of its properties has already been reported (Byfield and Scherbaum, 1961). In addition, two other laboratories have performed some interesting metabolic studies on Tetrahymena particles (primarily ribosomes) isolated by classical fractionation procedures (Mager and Lipmann, 1958; Plesner, 1961). The present brief communication deals with the isolation of mitochondria from Tetrahymena pyriformis and makes some observations on the points where their fractionation differs from the classical method. A complete survey of mitochondrial activity including phosphorylation and their role in protein synthesis is currently being carried out by the authors and will be reported presently. The presence of mitochondria in Tetrahymena had already been observed in electronmicrographs (Sedar and Rudzinska, 1956) and their cytological changes observed during the synchronous division process (Elliot, Kennedy and Bak, 1962).

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In the experiments described below the organisms were grown axenically in 1200 ml of medium in Fernbach flasks held at 18° C constant temperature; the growth medium has been described previously (Scherbaum, Chou, Seraydarian and Byfield, 1962). The organisms were harvested in the early stationary phase of growth by low speed centrifugation. They were washed twice in tap water to remove any remaining growth medium and then suspended in 9 times their volume of cold 0.25 M sucrose solution. All subsequent procedures were carried out in ice-water baths using pre-chilled equipment or as otherwise indicated. The suspension of organisms was then passed through a French press and observed microscopically to insure complete disruption of the cells. The resulting homogenate was divided into equal amounts where several samples were required for comparative purposes. It was then subjected to the differential centrifugation procedure following the methods described by Schneider (Schneider, 1957). Mitochondria were assayed for metabolic activity using the acetoacetate method (Walker, 1954). This method has proven useful and reproducible in our laboratory as it has for others (Weinbach and Garbus, 1956).

Using the methods described above it was soon observed that the mitochondria isolated were active but represented only part of the total activity present in any given sample. These observations led to an analysis of the sequential sedimentation of the mitochondria during increasing periods of centrifugation. The results of this survey are presented in Figure 1 in which mitochondrial activity is represented as μmoles of acetoacetate produced using the standard reaction mixture of Walker (Walker, 1954). P represents pellet activity while S indicates that remaining in the supernatant. The accumulation of RNA in the pellets is also included since in this range of the sedimentation it represents the appearance of ribosomes. In this typical experiment, which has been repeated several times, the sample was divided into equal portions immediately following homogenization. At the end of the experiment total protein in all samples (pellet plus supernatant) remained constant.

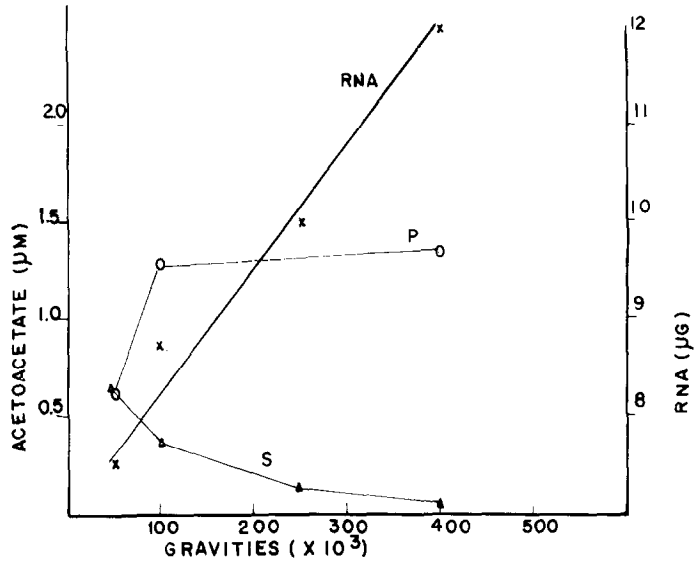

Figure 1

In pellets obtained from centrifugation periods of less than 50,000 total gravities the total mitochondrial activity was approximately constant and equal to that found in the other samples. However, separation of the pellet from the supernatant could not be performed with reproducible results. In pellets of 50,000 gravities and above the mitochondrial activity increased as is seen on the graph. When expressed as activity per mg of protein, however, a roughly sigmoid curve resulted with a peak at 250,000 gravities. This is not included on the graph for clarity's sake. It is believed that this result can be attributed to a more or less independent sedimentation of protein in the early and later stages of mitochondrial sedimentation. In the early phase this is probably due to cell debris and in the later stages to the rapid appearance of ribosomal protein. The activity is "purest" (when expressed in this manner) at 250,000 gravities because contamination is least compared to mitochondrial accumulation. The rapid increase in pellet RNA in the later stages (250,000 gravities and beyond) correlates with our previous observations that Tetrahymena contain large ribosomes with sedimentation coefficients of

S_{20} 125 or greater (Byfield and Scherbaum, 1961). Supernatant mitochondrial activity is barely detectable by the acetoacetate method beyond 400,000 gravities

From the results above it is concluded that the best mitochondrial fraction can be obtained from Tetrahymena by removing the nuclei and cell debris (centrifuging 5,000 gravities) and then subjecting the resulting supernatant to 100,000 gravities. The resulting pellet is rich in active mitochondria and they are easily resuspended by gentle homogenization. They may then be further purified by repeating the sedimentation at 50,000 gravities. This method represents an initial total separating force of twice the magnitude used in the customary method for isolating mitochondria which has proved so successful in higher systems. However, it results in a mitochondrial pellet that includes a much higher yield of total cellular mitochondria and contains fewer ribosomes than would occur if longer periods are used. In addition, the resulting supernatant will itself contain less mitochondrial contamination for ribosomal preparations.

In view of the results of these experiments it can be seen that any conclusions drawn from studies on particulate systems derived from Tetrahymena pyriformis must be interpreted with caution, especially if the classical fractionation methods have been used since inter-particle contamination is inevitable and greater than might ordinarily be expected. The relative delay in the sedimentation of the mitochondria (perhaps related to their shape -- see Sedar and Rudzinska, 1956) and the rapidity with which ribosomes begin to appear make separation of these two components particularly difficult. It should be stressed that this includes studies on ribosomal properties as well as those of mitochondria and particularly their comparative functions.

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