In earlier studies,¹ we have described the preparation of two classes of deoxyribonucleoproteins (DNP), I and II, based on their solubility in the presence of mono- and divalent cations. Nucleoprotein extracts from a bacterium contained only DNP II.² Two types of nonmalignant mammalian cells contained DNP I, while several types of malignant cells contained both DNP I and II fractions.¹ ³ We have extended these studies to nuclear extracts of Tetrahymena pyriformis in an attempt to determine the presence or absence of these DNP fractions in cells of different origin.

Materials and Methods.—Tetrahymena pyriformis GL was grown in a medium that consisted of 2 per cent (w/v) proteose peptone, Difco, and 0.2 per cent (w/v) yeast extract, Difco, at 26°. The cells were harvested at the early stationary phase and washed free of medium with a 0.005 M phosphate buffer, pH 7.0, containing 0.047 M NaCl and 0.001 M MgSO₄ (inorganic medium), and were then resuspended in inorganic medium to give a cell count of about 2–4 × 10⁶ cells/ml. After trying several variations, the following optimal conditions were used for the isolation of nuclei. In some aspects the following method is similar to that of Lee and Scherbaum.⁴ The cells maintained in the inorganic medium were ruptured by dropwise addition of Triton X-100 (a nonionic detergent) to a final concentration of 1 per cent (w/v) with gentle agitation of the cell suspension at room temperature. Within 5 minutes, almost all cells were completely lysed, and all further operations were carried out at 2–4°. The nuclei were sedimented at 1000 × g for 5 minutes and suspended in 0.25 M sucrose–0.0018 M CaCl₂. This step was repeated once more. The nuclear suspension showed practically no unruptured cells and very few cytoplasmic contaminants as identified by phase-contrast microscopy.

Prior to DNP extraction, the nuclei were washed twice with 0.15 M NaCl–0.015 M trisodium citrate, pH 7.7, in order to remove any divalent metal ions, which promotes the final dissolution of the nuclei. The sediment was washed once with 7 × 10⁻⁴ M phosphate buffer, pH 7.7, during which step considerable swelling of the nuclear mass was observed. The mass was suspended in about 10 vol of additional phosphate buffer, extracted overnight, clarified by centrifugation at 35,000 × g for 30 minutes, and dialyzed against phosphate buffer for 18 hours.

Solubility of DNP in salt solutions was determined by a previously described method.¹ The method in brief consisted of adding an equal volume of a salt solution to an appropriately diluted nuclear extract, removing any precipitate formed by centrifugation and using the absorbancy of the supernatant at 260 mμ as an index of the amount of DNP precipitated.

DNA was determined by the diphenylamine reaction.⁵ RNA was separated by the method of Ogur and Rosen⁶ and measured by the Orcinol method.⁵ Protein was determined by the biuret reaction.⁷
Results and Discussion.—Nuclear extracts were colorless and faintly opalescent, and contained about 1 mg of DNA, 6–8 mg of protein, and 0.12–0.15 mg of RNA. Ultraviolet absorption spectrum of the extract showed a maximum at 258 m\(\mu\) and a minimum at 242 m\(\mu\).

The solubility curves of DNP in NaCl and Mg acetate are depicted in Figure 1. Earlier studies have shown\(^1\) that the nature of the anion does not influence the solubility of DNP at neutral pH. Progressively increasing concentrations of salt yielded reversing profiles characteristic of mammalian DNP.

![Figure 1](image)

FIG. 1.—The solubility profiles of deoxy-ribonucleoproteins in the presence of mono- and divalent cations.

Examination of data as shown in Figure 1 indicated the presence of two classes of DNP: saline-insoluble (DNP I) and saline-soluble but 0.01 M Mg acetate-insoluble (DNP II) fractions. In a typical experiment it was shown, based on the DNA content, that 80 per cent of DNP was precipitated at 0.15 M NaCl and 100 per cent at 0.01 M Mg acetate. The absorbancy of the supernatant at 0.01 M Mg acetate was ascribed primarily to ribonucleoproteins. DNP I and II were precipitated from nuclear extract by serial addition of NaCl and Mg acetate at salt concentrations at which each showed minimum solubility. The protein to DNA ratio for DNP I was 1:1 and for DNP II 1:4. While no RNA was detected in DNP I, it constituted about 10 per cent of total nucleic acids in DNP II.

On comparison of solubility studies including Tetrahymena DNP preparations with those of other DNP’s, the following pattern emerges. Calf thymus and human term placenta contained only DNP I, while extracts from four types of malignant cells, including (a) Ehrlich ascites tumor cells, (b) hepatoma 134 ascites tumor cells, (c) granulocytic leukemic cells, and (d) hydatidiform mole, yielded both DNP I and DNP II as did Tetrahymena cells. On the other hand, a bacterial nucleoprotein extract was only composed of DNP II.\(^1^—^3\)

The presence of DNP II observed in bacterium (Bacillus subtilis), protozoan, and certain mammalian tumor cells, and its absence in two nonmalignant mammalian tissues is probably more than coincidental.

This study is being extended to other types of cells in order to determine whether or not the presence of saline-soluble DNP is a characteristic of malignant and undifferentiated cells.

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