Incorporation of Thymidine in the Cytoplasm of *Amoeba proteus*.*

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While a considerable body of information on the synthesis and localization of ribonucleic acid (RNA) in *Amoeba proteus* has been accumulated in recent years, very little is known about desoxyribonucleic acid (DNA) in this organism. In the course of numerous autoradiographic experiments with P^{32}, C^{14}-adenine, and C^{14}-orotic acid (1) RNA was found to be the only demonstrably labelled, acid-insoluble constituent of the cells. The faintness of the Feulgen reaction in the amoeba nucleus suggests low concentration of DNA as a plausible explanation for the absence of detectable precursor incorporation into this compound. The recent availability of high specific activity tritium-labelled thymidine offered a means of circumventing this difficulty. We have consequently incubated amoebae with this precursor and looked for autoradiographic evidence of incorporation into nuclear DNA. Such evidence has now been obtained. However, contrary to expectations, we have also found extensive cytoplasmic labelling in all of the cells examined. This note is intended to describe the latter finding and to attempt its preliminary evaluation.

Methods

Two groups of *Amoeba proteus* cells, one taken from a culture recently fed with *Tetrahymena pyriformis*, the other from a culture which had been starved for 7 days, were washed in a penicillin solution (100 units per ml. of amoeba medium) and placed in standard amoeba medium (2) containing 10 microcuries of radiopure tritiated thymidine (360 mc./mM, Schwarz Laboratories) per milliliter. The cells were incubated in the labelled medium at 17–18°C. for 44 hours and at the end of the incubation period were washed free of external radioactivity, placed on albumenized microscope slides, and fixed and flattened with a coverslip bearing a small drop of 45 per cent acetic acid. After removal of the coverslip by freezing over dry ice, the slides were passed through ethanol-acetic acid (3:1), two changes of 100 per cent ethanol, two changes of 95 per cent ethanol, and finally air dried.

Of each set of cells, pre-starved and pre-fed, five slides were prepared, each with 10 to 20 amoebae. The cells on three of these slides were subjected to enzyme digestion with: (*a*) desoxyribonuclease (Worthington, 0.5 mg./ml. in one-quarter strength McIlvaine's buffer at pH 7.0 with a trace of $MgSO_4$ at 38°C. for 4 hours); (*b*) ribonuclease (Worthington, 0.3 mg./ml. in distilled water, pH adjusted to 7.0 with Na_2HPO_4, at 38°C. for 4 hours); (*c*) desoxyribonuclease followed by ribonuclease. All slides were washed and covered with Kodak Ltd. AR-10 emulsion according to the method first described by Doniach and Pelc (3). The preparations were developed after 77 days of exposure in the dark at 5–7°C. Analysis consisted of locating the cells by phase contrast microscopy and examining the overlying autoradiographic emulsion with both brightfield and darkfield illumination.

RESULTS

With the exception of the cells subjected to digestion with desoxyribonuclease, all of the amoebae examined showed radioactivity in the cytoplasm. There was no obvious difference in the amount of cytoplasmic labelling between pre-fed and pre-starved cells. Nuclear labelling was found in only a few cells of both pre-fed and pre-starved cultures. While the ribonuclease-digested cells appeared to be labelled as extensively as untreated cells, the amoebae which had been subjected to desoxyribonuclease digestion or to digestion with both desoxyribonuclease and ribonuclease showed either no labelling or only a slight, barely detectable residue of radioactivity. A few of the cells and their autoradiographs are shown in Figs. 1 to 3.

DISCUSSION AND CONCLUSIONS

It is difficult to avoid the conclusion that the labelled component we have described is DNA. The specific precursor used, the acid insolubility of the labelled entity, and its complete or near complete removal with desoxyribonuclease all point in that direction. The possibility that the tritiated thymidine has undergone some conversion and become incorporated into the amoeba's

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RNA is argued against by the apparent inability of ribonuclease to solubilize the labelled component and by the fact that only a fraction of the cells showed labelled nuclei; all of the RNA precursors studied in the past have resulted in strong labelling of all the nuclei in a population of amoebae incubated for a similar period of time. In short, the cells we have analyzed appear to contain DNA in the cytoplasm.

The most striking aspect of the cytoplasmic labelling is its uniform occurrence in all of the cells studied. Incorporation of thymidine in the cytoplasm seems to be uninfluenced by the state of nutrition of the amoebae. While the density of the autoradiographic grain pattern varied to some extent from cell to cell, there appears to be no consistent difference in this respect between the pre-starved cells and those which had been fed just prior to incubation with thymidine. It must be kept in mind, however, that the precision of quantitative comparisons is kept very low by the short range of the tritium beta particles. We cannot rule out the possibility that subtle quantitative differences in incorporation do exist between the pre-starved and the pre-fed cells.

The density of the autoradiographic images, when related to the long exposure period used, suggests that the absolute amount of incorporation of tritiated thymidine into DNA in the cytoplasm is very low and might well escape detection in experiments designed to look at nuclear DNA metabolism. The Feulgen reaction does not reveal any DNA in the cytoplasm of *Amoeba proteus* other than that in the nuclei of partially digested *Tetrahymenae* concentrated in food vacuoles. We may thus conclude that the absolute amount of DNA in the amoeba cytoplasm is very small. Existing evidence on the stability of DNA (4 to 7) suggests further that the incorporation we have observed reflects synthesis of new DNA rather than direct exchange of thymidine. It is still questionable, however, whether the data on DNA stability are sufficiently precise to rule out completely the possibility that the small amount of incorporation we are dealing with could not at least in part be due to exchange. The strongest argument against exchange as a major contributing factor in the present experiment is the fact that the incorporation we have observed is not dependent on DNA concentration: most nuclei were not detectably labelled despite the fact that nuclear DNA is sufficiently concentrated to give a positive Feulgen reaction while the postulated cytoplasmic DNA is not.

The main questions raised by the observation of cytoplasmic DNA are those inquiring into its origin and its possible function in the physiology of the cell. The absence of a demonstrable difference between pre-fed and pre-starved cells makes it unlikely that digestive residues of *Tetrahymeae* are directly concerned with the cytoplasmic DNA labelling. Nor does it seem very probable that the labelled cytoplasmic DNA comes from the amoeba's nuclei; if this were the case, nuclear DNA labelling should have been more prevalent. (A more critical evaluation of this possibility will be made after the completion of current incorporation experiments with anucleate fragments.) The two most likely interpretations which suggest themselves to us are (*a*) we are dealing with a cytoplasmic infective agent which may or may not be characteristic of *Amoeba proteus*, or (*b*) we are observing a normal cytoplasmic process of DNA synthesis, possibly employing a cytoplasmic enzyme of the type recently reported for mammalian cells (8, 9) which had previously escaped detection in intact cells because of its low concentration and rate. While it may not be possible or even necessary to distinguish between a ubiquitous infective agent and a normal cytoplasmic component, we can find out whether or not the phenomenon described is restricted to the particular strain of cells used in the present experiment. It is also within the realm of the experimentally approachable to ask whether the cytoplasmic DNA plays a role in the amoeba's nuclear DNA metabolism or whether it should be considered an independent cytoplasmic component. We are obviously in need of further experimental evidence before a full interpretation of our findings is possible.

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Explanation of Plate 427

PLATE 427

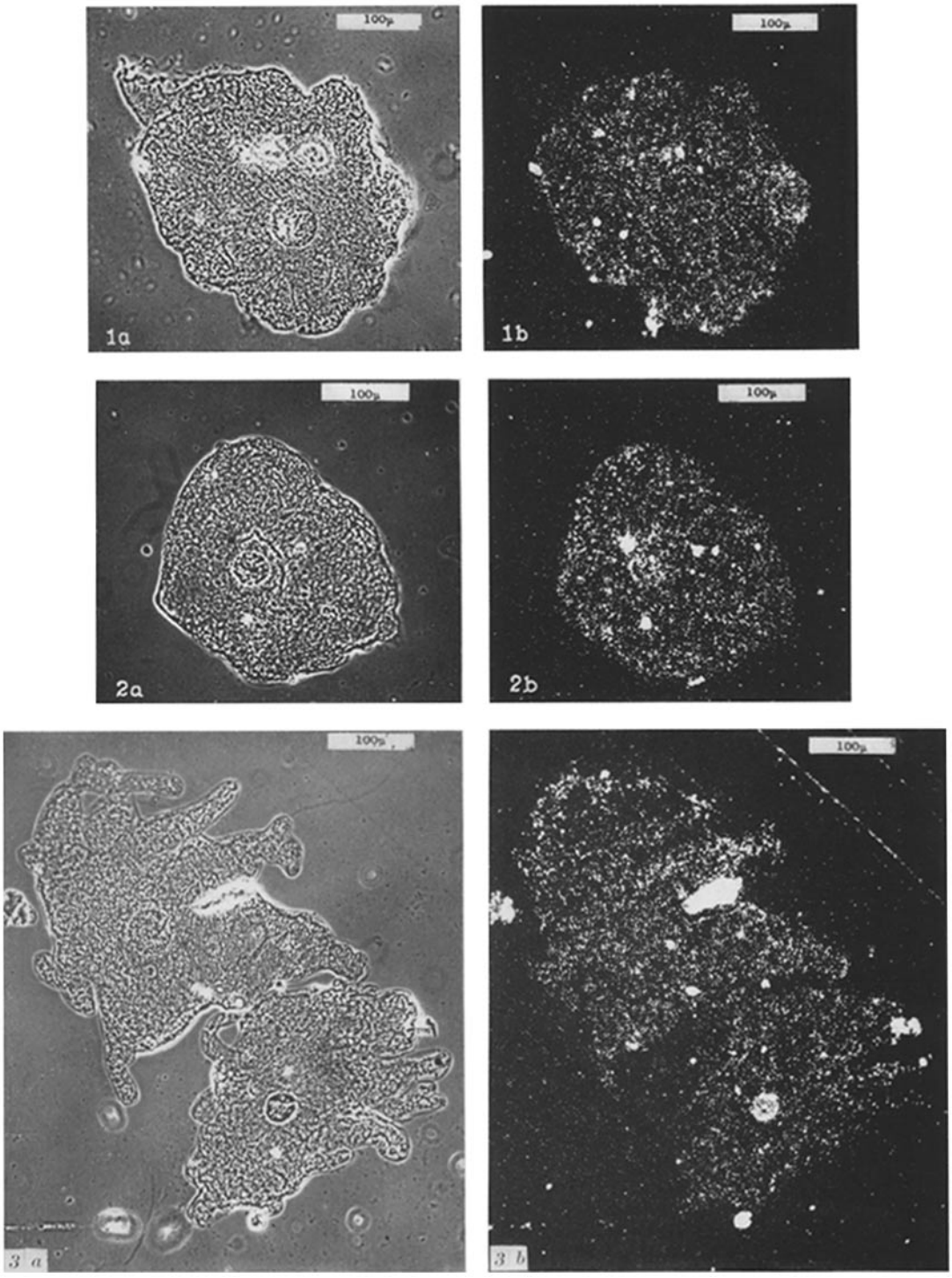
FIGS. 1 to 3 represent phase contrast (*a*) and darkfield (*b*) photographs of amoebae and the corresponding autoradiographic images.

FIG. 1. A cell which had been fed just prior to incubation with tritiated thymidine.

FIG. 2. A cell which had been starved for 7 days prior to incubation with tritiated thymidine.

FIG. 3. Two cells which had been fed just prior to incubation with tritiated thymidine and which were subjected to ribonuclease digestion before autoradiography. (The object which appears at the right margin of the upper cells in both phase and darkfield photographs is non-radioactive debris.)

See text for interpretation.



(Plaut and Sagan: Thymidine in cytoplasm of *A. proteus*)