G. L. Hoffman, R. A. Duce, E. J. Hoffman, *J. Geophys. Res.* 77, 5322 (1972).
14. G. E. Gordon, W. H. Zoller, E. S. Gladney, paper presented at the 7th Annual Conference on Trace Substances in Environmental Health, University of Missouri, Columbia, June 1973.
15. Supported in part by the National Science Foundation, Office of Polar Programs, under grant GV-33335 and by National Aeronautics and Space Administration grant NSG-398 to the Computer Science Center of the University of Maryland. Support for the fieldwork was supplied by Dr. C. Ponnamperuma through National Aeronautics and Space Administration grant NGR-21-002-317. We thank E. Bailey who helped in the analysis of some of the samples and the staff of the National Bureau of Standards reactor for carrying out the neutron irradiations. We also thank Prof. M. Magnusson, director of the Science Institute, University of Iceland, and Dr. S. Hermannsson, director of the National Research Council of Iceland, for their invaluable assistance. This work is a portion of a Ph.D. dissertation by E.J.M. in chemistry at the University of Maryland.

18 September 1974; revised 20 January 1975

Tetrahymena: Growth Without Phagocytosis

Abstract. *We have succeeded in growing a* Tetrahymena *mutant without food vacuoles in growth media supplemented with vitamins and heavy-metal salts. This finding implies the existence of adequate alternative routes of entry for every required nutrient, and clearly indicates that the food vacuole in* Tetrahymena *is a dispensable cellular organelle. The growth of the mutant without food vacuoles makes available a valuable experimental tool.*

Tetrahymena cells can take up particulate nutrients by phagocytosis, that is, the formation of food vacuoles, at a single, specialized location on the cell surface, the oral apparatus. The role of the food vacuole has posed a problem since the introduction of defined growth media composed of soluble nutrients. Carrier-mediated transport systems have been demonstrated in *Tetrahymena* (*1*). At least some of these systems are thought to be located at sites other than the food vacuole membrane (*2, 3*), possibly in the plasma membrane.

In the nutrient media commonly used to grow *Tetrahymena*, food vacuole formation appears to be essential (*3, 4*). However, in the presence of glucose and high concentrations of nucleosides, cells of strain GL of *T. pyriformis* can grow while forming vacuoles at a very low rate (*5*). This has led to the hypothesis that *Tetrahymena* cells can take up adequate amounts of all essential nutrients through a route or routes other than the food vacuole.

With the isolation of a mutant of *T. pyriformis* with a heat-sensitive capacity to form food vacuoles (*6*), it has become possible to test rigorously whether or not alternative routes of nutrient uptake exist and how important they are. This mutant grows and forms food vacuoles at normal rates at 30°C, but at 37°C the mutant cells are unable to form a functional oral apparatus, do not form food vacuoles, and stop growing in the commonly used growth media.

We have now succeeded in growing the mutant at 37°C in a 2 percent proteose peptone medium, supplemented with the high concentrations of vitamins and heavy-metal salts described in the legend of Fig. 1. Under these conditions, we have observed generation times as short as 3.5 hours for the mutant, and about 2 hours for the wild-type parental strain. One culture of the mutant has been maintained continuously under these conditions for an estimated 350 generations (by daily subculture) without a decrease in the growth rate. The metals in the medium precipitate at these high concentrations, but precipitate formation can be greatly diminished, if not eliminated, by the prior addition of 2 m*M* Na citrate, without any effect on the growth rate of the cells.

The same supplements enable the mutant cells to grow at 37°C in a chemically defined medium. Growth curves of the mutant at 37°C and the appropriate controls are shown in Fig. 1. Some of the cells in the medium show morphological abnormalities, and this medium may still be susceptible of improvement.

We have repeatedly verified that the mutant cells growing at 37°C in either medium do not form food vacuoles, both by direct observation under phase-contrast microscopy and by the addition of particles of India ink, as previously described (*6*). The mutant cells grown at 37°C also still show the morphological abnormalities of the oral apparatus revealed by silver impregnation (*6*). Thus the cells that grow are not revertants that have regained the capacity to form food vacuoles at 37°C. Such revertants, which can be easily detected by these tests, can be induced with nitrosoguanidine (*7*), and have very infrequently arisen spontaneously in the course of this study.

We assume that the mutation which affects the development of the oral apparatus at 37°C has not coincidentally increased nutrient-uptake capacities in the mutant. Thus we assume that the points discussed below apply equally well to the wild-type strains.

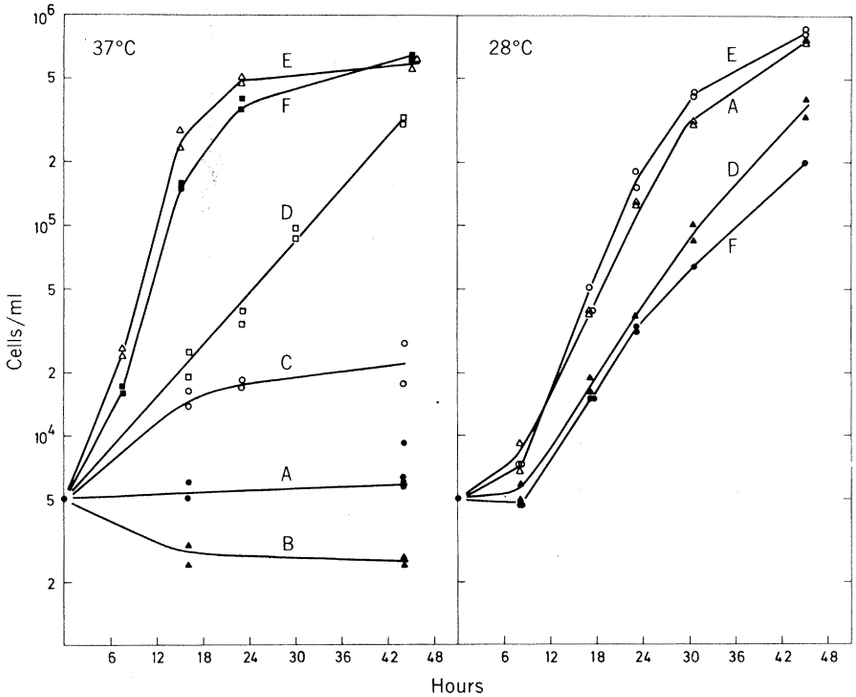

Fig. 1. Growth of mutant and wild type strains at 37°C (left panel) and 28°C (right panel) in defined media. The "basal" defined medium (BDM) has the composition described in table 1 of (*3*). The VIT solution adds 1 mg/ml of each of the nine vitamins already present in the medium (*11*). The HM solution adds each of the six heavy-metal salts already in the medium, at 25 times higher concentration (*12*). Curve A, mutant in BDM; B, mutant in BDM + VIT; C, mutant in BDM + HM; D, mutant in BDM + VIT + HM; E, wild type in BDM; F, wild type in BDM + VIT + HM. Duplicate cultures were incubated without shaking in 125-ml erlenmeyer flasks containing 10 ml of medium, and were inoculated from early stationary-phase cultures grown as follows: mutant at 37°C: BDM + VIT + HM at 37°C; all others: BDM at the corresponding temperature.

The results clearly show that *Tetrahymena* cells can take up sufficient amounts of all of their required nutrients without food vacuole formation, and more rigorously confirm previous work leading to the same conclusion (*5*). Carrier-mediated active transport (*1*), presumably through the plasma membrane, must contribute at least in part to this uptake without food vacuoles. Additionally, there may be micropinocytosis (*8*), but this mechanism has not been conclusively demonstrated in *Tetrahymena*.

The results also show that the food vacuole in *Tetrahymena* is a *dispensable organelle*. Even though it is essential for an adequate uptake of at least some vitamins and metals in the growth media commonly used, it must be concluded that the food vacuole makes no obligatory contribution to any vital cellular activity in *Tetrahymena*. Among other things, this excludes the possibility that the large amount of membrane processed during food vacuole formation (*9*) plays any obligatory role in plasma membrane accretion, or in the excretion of any cellular degradation products.

The two *T. pyriformis* strains studied, GL and the mutant of syngen 1 used here, differ with regard to the nutrients that must be present in high concentration to have growth without food vacuoles. The basis for this difference is not yet clear, but it could be temperature or strain differences (or both) in nutrient uptake rates. The two strains may well be as unrelated as if they belonged to different genera among mammals (*10*).

The growth media described here in combination with the mutant make available healthy *Tetrahymena* cells which do not form food vacuoles. These cells should be useful experimental material for dissecting out the contribution of food vacuoles to nutrition, excretion, and possibly other cellular functions, and should also more clearly expose other cellular systems involved in the exchange of materials between the cells and the medium. Additionally, these media allow the growth of cells (mutant or wild type) under essentially particle-free conditions.

LEIF RASMUSSEN
EDUARDO ORIAS*
Biological Institute of the Carlsberg Foundation, 16 Tagensvej, DK-2200, Copenhagen N, Denmark

References and Notes

1. P. B. Dunham and D. L. Kropp, in *Biology of Tetrahymena*, A. M. Elliott, Ed. (Dowden, Hutchinson & Ross, Stroudsburg, Pa., 1973), p. 165.
2. E. K. Hoffmann and L. Rasmussen, *Biochim. Biophys. Acta* **226**, 206 (1972).
3. L. Rasmussen and L. Modeweg-Hansen, *J. Cell Sci.* **12**, 275 (1973).
4. L. Rasmussen and T. A. Kludt, *Exp. Cell Res.* **59**, 457 (1970).
5. E. K. Hoffmann, L. Rasmussen, E. Zeuthen, *J. Cell Sci.* **15**, 403 (1974); L. Rasmussen, *Exp. Cell Res.* **82**, 192 (1973).
6. E. Orias and N. A. Pollock, *Exp. Cell Res.* **90**, 345 (1975).
7. G. B. Silberstein, E. Orias, N. A. Pollock, *Genet. Res.*, in press.
8. A. M. Elliot and G. L. Clemmons, *J. Protozool.* **13**, 311 (1966); R. D. Allen, *ibid.* **14**, 553 (1967); J. R. Nilsson, *C.R. Trav. Lab. Carlsberg* **39**, 83 (1972).
9. C. Chapman-Andresen and J. R. Nilsson, *C.R. Trav. Lab. Carlsberg* **36**, 405 (1968); T. R. Ricketts, *Exp. Cell. Res.* **66**, 49 (1971).
10. S. L. Allen and C. I. Li, *Biochem. Genet.* **12**, 213 (1974).
11. Nicotinic acid; Ca *d*-pantothenate; thiamine · HCl; riboflavin-5′-P · Na; pyridoxamine · 2HCl; pyridoxal · HCl; biotin; DL-6-thioctic acid; and Ca folinate.
12. Final concentration, in milligrams per 100 milliliters of medium: $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 35; $ZnSO_4 \cdot 7H_2O$, 11; $MnSO_4 \cdot 4H_2O$, 4.0; $CuSO_4 \cdot 5H_2O$, 0.75; $Co(NO_3)_2 \cdot 6H_2O$, 1.25; and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.25.
13. We thank Professor Erik Zeuthen for critically reading the manuscript, and Mrs. B. Dohn and J. D. Orias for excellent technical assistance. Partial support by NIH grant GM 21067 (to E.O.) is gratefully acknowledged.
* Permanent address: Department of Biological Sciences, University of California, Santa Barbara 93106.

28 April 1975

Electron Microscopy of Albumin Synthesis

Abstract. *Albumin molecules appeared to be synthesized in the light hepatocytes of rats by bound polysomes on rough endoplasmic reticulum and the nuclear envelope. The molecules were discharged directly into the cytosol, then to the external cellular spaces. This conclusion failed to support the current theory from biochemical studies that albumin is synthesized by bound ribosomes, discharged into the cisternae of rough endoplasmic reticulum, and transported to the smooth endoplasmic reticulum and then to the Golgi apparatus. In addition to the liver, positive synthetic activities were observed in the aorta, kidney, and hepatoma cells of rat. Earlier investigators have reported that only liver cells can synthesize albumin.*

The question of which cells synthesize albumin and how albumin is synthesized and distributed intracellularly has not been satisfactorily answered. Our immunocytochemical studies of these problems failed to support the current concept on the mode and site of albumin biosynthesis.

For preparation of Fab-peroxidase conjugate, purified rat albumin was injected into rabbits to produce antiserum. Rabbit immunoglobulin G (IgG) was separated from the antiserum by ion-exchange chromatography on QAE-Sephadex A-50 followed by rat albumin immunoadsorbents (*1*). After papain digestion of IgG (*2*), the Fab fragment was purified by Sephadex G-100 and rat albumin immunoadsorbents. Finally, the Fab fragment was conjugated with horseradish peroxidase by glutaraldehyde (*3, 4*). For tissue preparation, tissue slices from the rat liver, kidney, aorta, and the Chang rat hepatoma (*5*) were fixed in a mixture of formaldehyde and glutaraldehyde (*6*). The fixed nonfrozen tissues were sectioned with a Vibratome at 20 to 40 μm. The desirable sections were selected for incubation in Fab-peroxidase conjugate solution, then treated first with 3,3′-diaminobenzidine (*7*) and then with 1 percent osmium tetroxide. After dehydration, the sections were flat-embedded with special molds and Teflon-treated cover glasses (*8*) in Spurr embedding medium (*9*) for electron microscopy.

Under the light microscope, the albumin-synthesizing cells contained the brown reaction product which was produced by the antigen-antibody reaction between Fab-peroxidase conjugate and albumin. These positive cells were seen scattered in the liver lobules (Fig. 1a). Under the electron microscope, the active cells were identified as "light" hepatocytes, lipocytes, interstitial cells, as well as the endothelial cells of sinusoids and central veins. The "dark" hepatocytes and Kupffer cells contained no reaction product. The most intense reaction product appeared on the polysomes which were bound either to rough endoplasmic reticulum or the nuclear envelope (Fig. 1, b to d). Free polysomes played a lesser role in albumin synthesis. On occasion, albumin molecules were seen extended from bound polysomes into the cytosol, indicating, perhaps, a discharging process. In the cytoplasm, albumin masses were randomly distributed. They tended to adhere onto the surfaces of many cellular organelles such as mitochondria, peroxisomes, lysosomes, and the Golgi apparatuses (Fig. 1c). No albumin molecules were seen in the cisternae of nuclear envelope, rough endoplasmic reticulum, smooth endoplasmic reticulum, or Golgi apparatus in any cells observed so far. Therefore, albumin molecules appeared to be synthesized mainly by bound polysomes, discharged directly into the cytosol, and secreted then into extracellular spaces. Albumin molecules have been seen in the spaces of Disse, sinusoids, and bile canaliculi. These observations failed to support the theory based on biochemical fractionation studies by others (*10, 11*) who have theorized that albumin is synthesized by bound polysomes, discharged into the cisternae of rough endoplasmic reticulum, and transported to smooth endoplasmic reticulum and then to the Golgi apparatus.