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.

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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.
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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.

In biochemical studies, Peters (*12*) reported that albumin is present in the supernatant of homogenates from which mitochondria had been removed and in all fractions derived from the cellular organelles, such as nuclei, lysosomes, mitochondria, and microsomes. Evidently, when albumin molecules are discharged randomly and directly into the cytosol of a cell, they become adherent to various cellular organelles, and so are included in each of the cell fractions.

Fab-peroxidase conjugate has been successfully used for intracellular localization of immunoglobulins in intact lymphocytes and plasma cells and tissue sections (*3, 13*) to show the intracisternal localization of immunoglobulins in endoplasmic reticulum, Golgi apparatus, and the nuclear envelope. Therefore, an absence of reaction product in the cisternae of these organelles in our study cannot be attributed to the impermeability of the Fab-peroxidase conjugate to the cytomembranes. The fact that

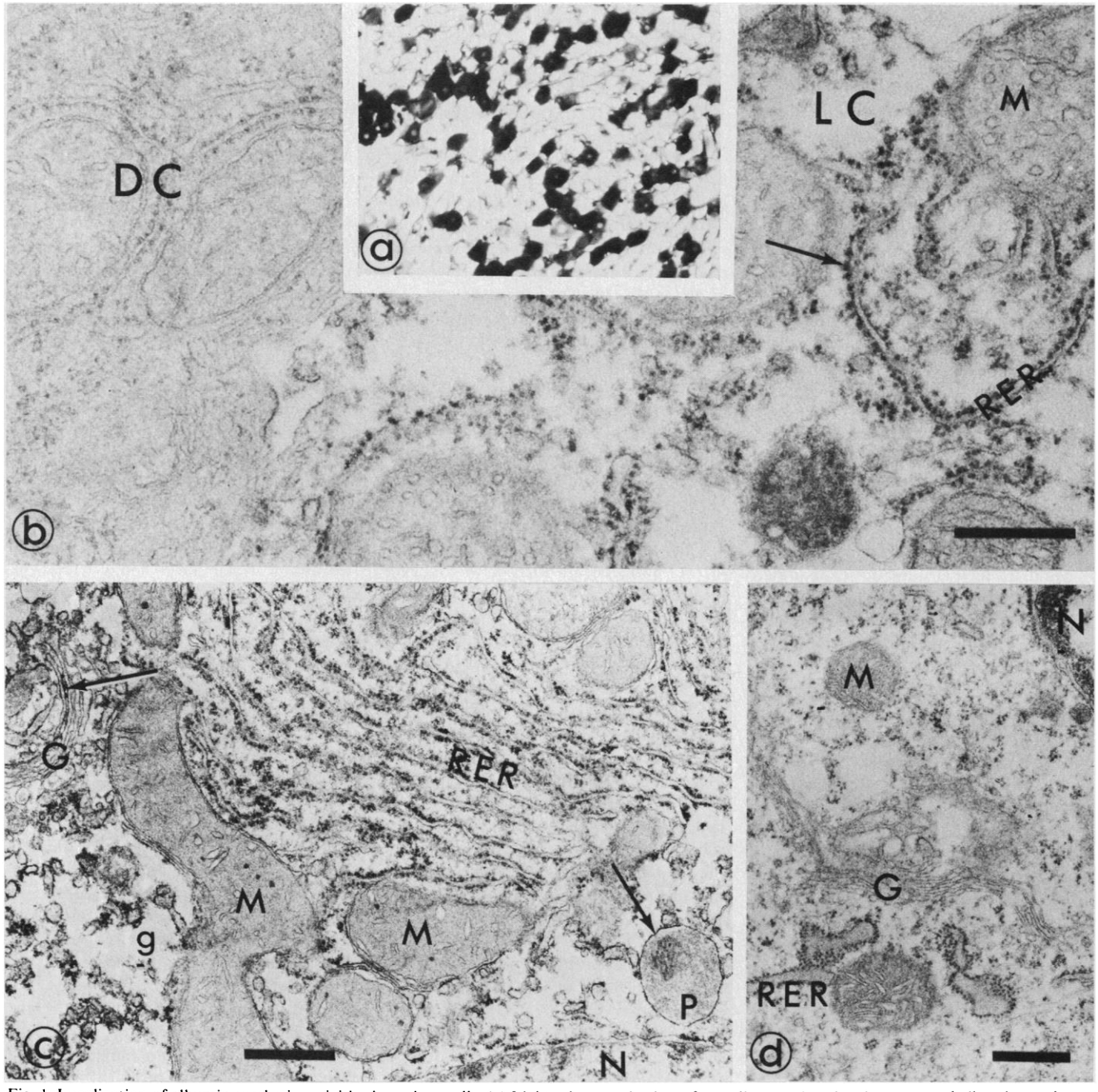

Fig. 1. Localization of albumin synthetic activities in various cells. (a) Light microscopic view of a rat liver section showing scattered albumin-synthesizing cells; not counterstained (× 360). (b to d) Electron micrographs of albumin-synthesizing cells. (b) Rat hepatocytes. The reaction product (arrow) is seen on the polysomes bound to rough endoplasmic reticulum (*RER*) in the light cell (*LC*). The dark cell (*DC*) on the left shows no comparable synthetic activity. The electron density of ribosomes and membranes in the dark cell is produced by staining with weak uranyl acetate. (c) Rat hepatocyte, showing an area of stacked rough endoplasmic reticulum with positive polysomes. The albumin molecules become adhered (arrow) to peroxisomes (*P*), mitochondria (*M*), and to some vesicles or portions of smooth endoplasmic reticulum near the Golgi apparatus (*G*). A small amount of reaction product is seen between, but not "in," the Golgi cisternae (arrow). Aggregated albumin molecules are also located in the glycogen area (*g*) which appears empty because of the unstainability of the glycogen molecules. Positive polysomes on the nuclear envelope of the nucleus (*N*) is shown at the lower right corner. (d) Rat kidney, Bowman's capsular epithelial cell, showing reaction product on rough endoplasmic reticulum, nuclear envelope, and in the cytosol. In the micrographs (b to d), note especially that no albumin molecules are seen in the cisternae of the rough endoplasmic reticula, nuclear envelopes, or the Golgi apparatuses (scale bar, 0.5 μm).

albumin contains no carbohydrate in its molecules (*11, 12, 14*) may render it unnecessary for albumin to be transported to the Golgi to acquire carbohydrate before its discharging into cytosol. Presumably, albumin belongs to a class of proteins (*15*) whose molecules are synthesized by bound polysomes and discharged directly into the cytosol.

To establish the specificity of the immunocytochemical reaction, some critical control experiments have been conducted. The binding of Fab-peroxidase conjugate with intracellular albumin did not occur when the conjugate was previously treated with excess rat serum albumin. Incubation of specimens with horseradish peroxidase alone did not produce reaction product. When Fab-peroxidase conjugate against potato acid phosphatase was used, no reaction product was present, except for the expected endogenous peroxidase activity in peroxisomes and erythrocytes. When tissue section was incubated with 2 percent H_2O_2 at room temperature for 10 minutes before incubation with Fab-peroxidase conjugate, the reaction product was present at the site of albumin synthesis, although endogenous peroxisomes showed negative reaction. The last experiment indicated that the reaction product was not due to endogenous peroxidase activity. Furthermore, since only some liver cells could synthesize albumin, the albumin-negative cell adjacent to the positive cell served as another excellent control.

In addition to the liver, positive albumin synthetic activity has been observed in other tissues, including aortic endothelium, renal vascular and lymphatic endothelium, Bowman's capsular epithelium (Fig. 1d), proximal convoluted epithelium, interstitial cells, and the ascitic cell of the Chang rat hepatoma. The intracellular sites of localization of albumin in these tissues were exactly the same as those in the hepatocytes. Previously, investigators believed that only liver cells could synthesize albumin (*14*).

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Insular Biogeography: Of Mice and Mites

Abstract. *The number of mite species using cricetid rodents in North America is related to the host distributional areas. The age and latitude of the distributional areas have unimportant effects on the number of mite species using a rodent species. The relation between species and area is analogous to species equilibrium numbers of island faunas.*

Terrestrial mammals often support a large and diverse assemblage of endo- and ectoparasites. We have examined the theories of island biogeography introduced by MacArthur and Wilson (*1*) and subsequently applied to insect communities of host plants (*2*) for their applicability to mice and mites.

Mites associated with mammals can be parasitic or phoretic. They range in size from the microscopic *Demodex* species (100 μm) to the relatively large and visible ticks (Ixodidae). The endoparasitic mites, ticks, and chiggers have been excluded from consideration, restricting the component community to those mites, found on the skin or hair of mammals, that either spend their entire lives on their hosts or remain on a mammal for a period of time sufficient for dispersal (*3*). Roughly three guilds, based on food preference (blood, tissue fluids, or hair) can be identified in this community with a fourth guild of phoretic mites (*4*). We have chosen those mites reported from North American cricetid rodents because they are well studied and represented by a large number of species. Distributional areas of the rodents were

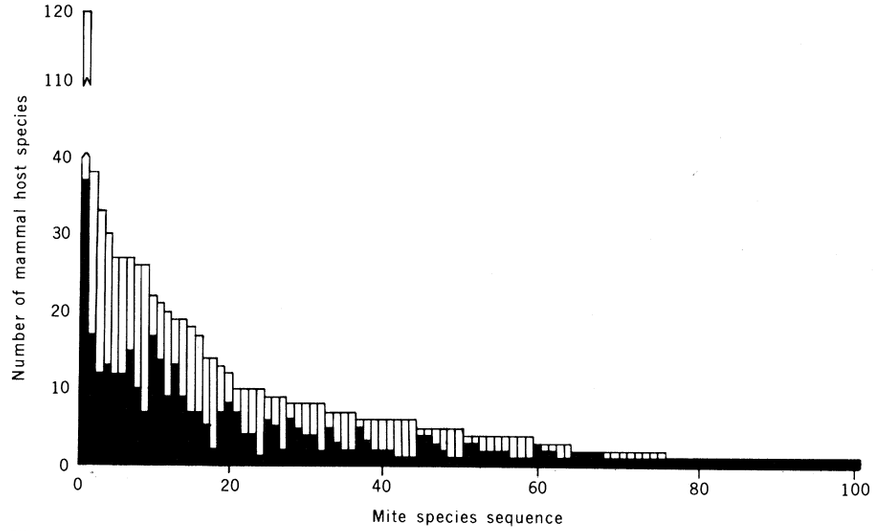

Fig. 1. Host specificities of mites found on cricetid rodents in North America. The ordinate is the number of mammal species used by a mite species. The abscissa represents each mite species ranked by the number of mammal species used as hosts, those with the fewest species farthest from the origin. The solid bars represent the number of cricetid species used; the hollow bars, all other mammal species used. One can conceive of the graph as ranking the mite species in order of increasing host specificity. [Based on data from Whitaker and Wilson (*6*)]