Subcellular Fractionation of *Tetrahymena pyriformis* by Zonal Centrifugation: Changes in Enzyme Distributions During the Growth Cycle

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Changes in the cytology of *Tetrahymena pyriformis* during the growth cycle, and the presence in this organism of vacuoles containing acid phosphatase were reported by Elliott & Bak (1964). Subsequently, five acid hydrolases were shown to be lysosomal and the distribution of these particles, as well as that of mitochondria and peroxisomes, after sucrose-density-gradient centrifugation was studied (Müller, Baudhuin & de Duve, 1966).

Exponential growth was biphasic and was followed by a stationary phase and a phase of decline. Cells were harvested at different stages of growth and were disrupted in a hand homogenizer. The whole homogenates were centrifuged at 10000 rev./min. for 2 hr. in a prototype HSA zonal rotor (kindly provided by M.S.E. Ltd.) or in a B-XIV rotor at 30000 rev./min. for 4 hr. (10000 g_{av} . and 44000 g_{av} . respectively).

The distribution of mitochondria in the fractions was determined by using succinate-ferriyanide oxidoreductase as a marker enzyme, that of peroxisomes by using catalase and that of lysosomes by using acid phosphatase and acid deoxyribonuclease. The median buoyant density of mitochondria was 1.211 and that of peroxisomes 1.216.

Acid phosphatase had the same distribution whether assayed with *p*-nitrophenyl phosphate or β -glycerophosphate as substrate. Distributions of acid deoxyribonuclease and acid phosphatase were similar throughout the growth cycle except that a part of the activity of the former enzyme, associated with very slowly sedimenting particles, was not present in homogenates obtained from cells harvested towards the end of the second phase of exponential growth.

Only one population of particles containing acid phosphatase was found in homogenates of cells up to the end of the phase of initial rapid exponential growth. A second, heterogeneous population of greater buoyant density and greater sensitivity to treatment with Triton X-100 then appeared, and its acid phosphatase activity relative to that of the first population progressively increased.

The relative distribution of NADH- and NADPH-cytochrome *c* reductases also changed during growth, but were always different from each other. A part of the former enzyme activity was associated with mitochondria. The subcellular

components isolated in the various fractions were also characterized by electron microscopy.

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Subcellular Localization of Reserpine and 5-Hydroxytryptamine in Blood Platelets after Reserpine-Induced Release

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Reserpine and certain other alkaloids of the Ranwolffia group possess the property of depleting monoamine stores both *in vitro* and *in vivo*. Opinions differ widely, however, on the precise chemical nature of this release and whether the action of the drug is directed towards irreversible blockade of a cell-membrane amine pump or to the inhibition of a Mg^{2+} -ATP-dependent uptake-storage mechanism in the intracellular granules (Carlsson, Hillarp & Waldeck, 1963). Blood platelets store 5-hydroxytryptamine (5HT), and reserpine at low concentrations will release the amine, one molecule of reserpine apparently liberating hundreds of molecules of 5HT (Carlsson, Shore & Brodie, 1957). As with other amine-storage tissues, the action of reserpine on the platelet is complex. Some experimental findings suggest inhibition of an active storage process with 5HT leaving the cell by diffusion (Hughes, Shore & Brodie, 1958). Under certain conditions, however, reserpine may also exert a conserving effect by decreasing passive 5HT outflow through the cell membrane (Bartholini, Da Prada & Pletscher, 1965). Previously we have described the subcellular localization of 5HT in blood platelets (Minter & Crawford, 1967), and these investigations have now been extended to the study of 5HT and reserpine localization within platelet fractions after reserpine-induced 5HT release.

Homogenates of platelets in 0.3M-sucrose have been fractionated by sucrose-density-gradient centrifugation and four major zones examined, A, B, C and D. The low-density fraction A consists of sheet and vesiculated membrane fragments. Fraction C includes the α -granules, 5HT-storage granules, mitochondria and other unidentified organelles. Fractions B and D are almost free from particulate material. Reserpine and 5HT have been measured by sensitive spectrophotofluorimetric procedures [reserpine (Glazko, Dill, Wolf & Kazenko, 1956); 5HT (Crawford, 1965)].

Incubation of platelets for various periods at 37°