

ON THE INCLUSIONS OF HANSENULA ANOMALA

GEORGES KNAYSI

*Laboratories of Bacteriology, New York State College of Agriculture,
Cornell University, Ithaca, New York*

Received for publication July 26, 1946

In the cell of *Hansenula anomala* it is possible to recognize two types of inclusions: lipoid inclusions and volutin granules. They are considered to be reserve materials. This may be said of volutin, which disappears in old cultures, but the evidence for the reserve function of the fatty inclusions rests chiefly on their quantitative sensitivity to the composition of the environment.

Hansenula anomala is especially suitable for studying the functions of inclusions, since it seems not to require nitrilites and grows readily in a medium of the following composition: ammonium sulfate, 0.2 g; glucose, 0.2 g; an equimolar mixture of monopotassium and dipotassium phosphate, 0.23 g; and distilled water, 100 ml. In this medium the yeast reproduces normally and deposits relatively considerable quantities of lipoid inclusions and volutin granules.

Most of the observations reported below were made on aerobic microcultures prepared with cells washed several times with sterile, distilled water. Lipoid inclusions were observed in the living cells, observations thus obtained were confirmed by staining with Sudan black B. Volutin was demonstrated by staining with methylene blue at pH 1.7.

Hansenula anomala grows, but to a limited extent and with considerable difficulty, in distilled water in which a viable cell usually buds several times; the buds separate from the mother cell but remain small. Observation of the living cells and cytochemical study show that the original lipoid inclusions of the mother cell are neither reduced in size nor do they pass into the bud during growth, but that one or more fatty inclusions are formed *de novo* in each bud; these may slowly increase in size after separation of the bud. On the other hand, growth is accompanied by gradual reduction in the volutin content of the mother cell.

In a solution of ammonium sulfate (0.2 g + 100 ml), the picture is qualitatively similar to that observed in distilled water. However, growth is noticeably faster and goes further than in distilled water, and the rate of disappearance of volutin is such that in 4 days at 25 to 28 C about 80 per cent of the cells are volutin-free.

Of considerable interest is the behavior of cells and inclusions in a solution of glucose (0.2 g + 100 ml). In this environment growth is rapid and extensive, so that a loopful of a heavy suspension of washed cells inoculated into 5 ml of the glucose solution produces turbidity within a day at 33 C. The new cells are ellipsoidal, volutin-free, and each contains at least one relatively huge lipoid inclusion; the protoplasm is hyalin. Such cells do not stain with methylene blue at a low pH, and stain only faintly at pH 4 to 7, so that, in these cells, it is

possible to observe the nucleus after fixation by heat and mounting in a dilute solution of methylene blue. However, they remain strongly gram-positive. When these cells are washed several times with distilled water, resuspended in distilled water, and incubated aerobically in microculture, the fatty inclusions become gradually smaller and often disappear. Their disappearance is accompanied by growth and by the reappearance of volutin in the form of small granules variable in number. Upon further incubation of the microculture, new lipid inclusions are formed. During the time these changes take place in the strongly aerobic microculture, there is no noticeable growth, decrease in size of the fatty inclusions, or formation of volutin in the suspension from which the microculture was prepared.

The observations reported above lead to the conclusion that volutin is readily utilizable by *Hansenula anomala* as a source of nitrogen, but with considerable difficulty as a source of energy. This probably means that volutin is hydrolyzed, that *d*-ribose is not utilizable as a source of energy, and that the purine and pyrimidine bases are suitable sources of nitrogen; the energy expended during the limited growth in distilled water may also have been derived from these bases.

It is also obvious that the lipid inclusions are noticeably utilizable only by volutin-free cells, in an environment devoid of nutrients, and under strongly aerobic conditions, both as a source of energy and a source of nitrogen. There is no evidence that either type of inclusions is used up during active growth in complete nutritive media; on the contrary, the rate of their formation in such media is highest during active growth.

Since under the proper conditions both types of inclusions may be formed in distilled water, and only by growing cells, and since one type may be transformed to the other, they could not be considered reserve material in the sense of Meyer (1912), i.e., materials "stored by the cell at one time so that they will be used up when external nutrients become relatively deficient, or when there is increase in cell growth, cell respiration, etc." They do not seem to be produced by special processes, but as by-products of normal metabolic processes involved in growth, and because of their insolubility, or limited solubility, they precipitate out in the cell. The transformability of one type to the other under the proper conditions suggests that they are by-products of related metabolic processes.

We have not yet investigated the behavior of the inclusions during sporulation.

REFERENCE

- MEYER, A. 1912 Die Zelle der Bakterien. G. Fischer, Jena.