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Attempts to Produce Encystment in Chilomastix.

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The life histories of mammalian intestinal flagellates, particularly with reference to the process of encystment, have been investigated with much less satisfactory results than have those of the intestinal amoebae. Practically nothing is known regarding the conditions producing encystment or excystment in these forms. The results here reported are largely negative, but it seems worth while to summarize them briefly since they show what conditions are incapable of producing encystment *in vitro,* and since, furthermore, this phase of the investigation is being abandoned.

Da Cunha and Muniz[1] are the only workers to report encystment of *Chilomastix mesnili* in culture; their successful results were obtained with ordinary blood agar and with N.N.N. medium, on which the flagellates encysted in great numbers after being grown for 48 hours at 37°C. Experimental production of encystment in *C. intestinalis* from the guinea pig has been reported by Hegner.[2] Washed cysts from guinea pigs were fed young chicks and apparently hatched in the digestive tract of the latter, since motile forms were discharged in cecal droppings 6 days later. Four days after this, cysts as well as trophozoites were abundant in the cecal droppings, but by the fourteenth day the infection had disappeared. The chick, in this instance, served as a living culture tube.

In a series of experiments running over several years, *Chilomastix mesnili* was cultivated successfully in a number of standard culture media, such as ovo-mucoid, Ringer-egg (with and without albumen), Locke-egg (with and without albumen), and Locke-egg-serum. In cultures continued over several months, there was no evidence of anything approaching a life cycle, such as any rhythmic variation in the division-rate; and no cysts were ever found. On Locke-egg-albumen, the flagellates appear able to grow and multiply indefinitely without producing cysts.

It was not possible to produce encystment of motile *Chilomastix in vitro,* despite numerous attempts to obtain it by modification of

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[1] Da Cunha, A. M., and Muniz, J., *C. R. Soc. Biol.*, 1927, **97**, 1777.

[2] Hegner, R., *Am. J. Hyg.*, 1929, **9**, 529; **10**, 33.

the above cultural conditions. The following modifications of the standard culture media were made with the aim of producing encystment. The viscosity of the medium was increased by gradual evaporation or by the addition of dilute agar, gelatin or gum arabic. The concentration of substances normally present in the feces was increased without producing an unfavorable preponderance of bacteria by the addition of purified indol or skatol. Purified cholesterol was also employed. Both sterile rice starch and sterile rice flour were added to the various culture media. Although ingested by the trophozoites, they did not bring about any marked increase in their numbers nor cause them to encyst. The results of da Cunha and Muniz could not be confirmed; N.N.N. medium did not prove satisfactory for the cultivation of *Chilomastix intestinalis* of the guinea pig, even when guinea pig blood was used in its preparation. Care must be taken to avoid the inoculation of cysts into this medium, lest the process be supposed to take place in the culture tube. Lastly, the cultures were brought to room temperature, either gradually, or abruptly, as would be the fate of organisms expelled from the digestive tract, with again negative results.

Hegner's results have been amply confirmed in the transfer of mammalian *Chilomastix* to young chicks. Rectal injections of motile trophozoites of *C. mesnili* and of *C. intestinalis* have been made into 126 young chicks, for the most part 24 to 48 hours old. In 39 of these, infection was established and maintained for 24 hours or more; chicks frequently continued to discharge trophozoites for at least 4 days; the longest period for which they remained infected was 8 days. The chick cecum, for a time at least, offers a particularly favorable environment for *Chilomastix,* these flagellates were found frequently in relatively greater numbers in the chick than in the original host. Conditions in the chick cecum are apparently more suitable for *Chilomastix* than for the Protozoa associated with it in the guinea pig; *e. g., Trichomonas* or *Balantidium,* since inoculation of material from the guinea pig cecum into the chick often results in the partial or complete disappearance of these forms and a temporary increase in the frequency of *Chilomastix.* In one instance, encystment occurred in the chick cecum, 4 days after the inoculation of trophozoites. The process of encystment in the chick, however, offers the same difficulty in determining the causes which bring it about, as it does in the normal host.