

**Internal factors in the response of amoebocytes to stimulation.****LEO LOEB and I. T. GENTHER.**

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In the preceding paper we have shown that the character of the amoeboid response of amoebocytes to various stimuli varies within a certain range in accordance with the character of the solutions with which we surround these cells. However, we found in certain cases that the same kind of solution did not in all experiments produce the same effect, and by varying the conditions of our experiments sufficiently, we could demonstrate that one of the principal causes of such lack of constancy in results consists in the difference in the age of the cells which we use.

By age of the cells we understand the time which elapsed between the beginning of the migration of the cells out of the piece and the testing of the amoebocytes. Young cells are 6 to 12 hours old, cells of medium age are 24 to 36 hours old, and old cells are two or more days old. In general, young cells have a greater tendency to the formation of thread and tongue pseudopodia, while in older cells the tendency to produce balloon and drop pseudopodia is greater. Furthermore, in older cells the tendency to produce under unfavorable conditions pathological formations like courts and pseudofertilization membranes is likewise greater than in young cells. These two factors, favorable or unfavorable character of the medium, and age of the cells, may therefore either mutually intensify, or, on the other hand, neutralize their effects.

To cite some examples: Young cells may in addition to balloons form some tongue and thread pseudopodia in a N/2 NaCl solution, while older cells tend to form exclusively balloons. As we stated in a previous paper, if we add N/1000 HCl in N/2 NaCl to amoebocytes, balloon formation occurs, and if this solution is replaced by a solution of N/1000 NaOH in N/2 NaCl tongue and thread pseudopodia are usually sent out after some

time. In a number of cases we found, however, that even pouring on of alkali may lead merely to the formation of balloons. Whether the one or the other of these two reactions occurs seems to depend upon the age of the cells. It is especially in older cells that the pseudopodia assume the abnormal character of balloons even in alkali. Young cells, on the other hand, may in as unfavorable a solution as an isotonic solution of N/1000 HCl send out at least some tongue pseudopodia, although balloons always predominate in this acid. More frequent is the formation of tongue pseudopodia in an isotonic solution of N/5000 HCl. Also in a solution of  $\text{NH}_4\text{Cl}$ , which in general is not favorable for the formation of tongue pseudopodia, some tongue pseudopodia may appear in very young cells. Likewise, in ammonia, tongue pseudopodia are more frequent in young than in older cells.

Young cells differ, therefore, in certain respects from older cells; the former behave as if their protoplasm was more condensed, more plastic and able to undergo these rhythmic changes in consistency and viscosity on which amoeboid movement seems to depend. On the other hand, the older the cells are the more fluid they have apparently taken up, the more flaccid and flat they tend to be, the less they are able alternately to contract and extend in different parts of their protoplasm, and completely to return to the original contracted state after previous periods of softening. In the course of time the cells have evidently been injured in the medium in which they move, probably as the result of the action of certain substances. Tentatively we may assume that in consequence of cell injury the resynthesis which normally follows the splitting of certain cell constituents remains incomplete, and thus substances which tend to prevent the elimination of fluid from the cell, continue to act within the amoebocytes.