

This Week's Citation Classic

Townes P L & Holtfreter J. Directed movements and selective adhesion of embryonic amphibian cells. *J. Exp. Zool.* **128**:53-120, 1955. [Departments of Biology and Anatomy, University of Rochester, Rochester, NY]

This paper describes a tissue disaggregation-reaggregation analysis of early amphibian embryogenesis. It has helped to establish the notion that directed cell movements and cell affinity are of major importance in organogenesis. [The SC[®] indicates that this paper has been cited over 145 times since 1961.]

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"As an undergraduate at Harvard, my interests in embryology were kindled by the late Leigh Hoadley. I subsequently had the good fortune to become a graduate student of the renowned Johannes Holtfreter who suggested I employ his disaggregation-reaggregation techniques to analyze gradients and fields in neurogenesis. The time and setting for the proposed study were optimal; Holtfreter had some years previously published his classic studies of 'Gewebeaffinität' and the proposed research was an extension of those studies.¹

"The laboratory was remarkably uncomplicated. Holtfreter performed his own experiments; no technicians, no large research grants, no complex apparatus.

"Equipment consisted of little more than a dry-heat sterilizer, simple culture vessels (furniture castor dishes), a few chemicals, glass needles, and microscopes. Even today, the entire inventory (except for microscopes) could be purchased for a few hundred dollars. There was no pH meter; estimates were made by indicator dyes. Graduate students were required to do their own photography. The drawings in our publica-

tion were made by Holtfreter, who is an artist of exceptional talent, from my crude sketches.

"My involvement consisted of four years of rather meticulous work at the microscope; operating in the spring when embryos were available, spending the rest of the year studying serial sections. Although the work had potential for tedium, the disaggregation-reaggregation phenomena were so remarkable that I found myself driven to try all conceivable tissue combinations. Holtfreter was the perfect mentor; interested, stimulating, supporting, and demanding of maximal effort in a firm but gentle way.

"I became interested in some biochemical aspects of cell aggregation and examined the effects of adenosine triphosphate (ATP) and metabolic inhibitors. I also extracted ricin from castor beans and found it agglutinated embryonic amphibian cells. These studies were omitted from our publication because they were deemed to be of little significance. Of interest is the important work which has since been done with concanavalin A. The need to denude large numbers of amphibian embryos prompted the use of trypsin and other proteolytic enzymes with the finding that the jelly layer and fertilization membrane are susceptible to proteolysis. This study was published with mention that trypsin and chymotrypsin 'selectively digested the amphibian coat resulting in cell disaggregation' and that the 'action on the coat will be considered more extensively in another context.'² However, these observations were considered to be of little significance and were omitted from our publication. Trypsin has since been widely used to produce tissue disaggregation.

"Although I have regrettably not continued these studies, I have followed with interest the publications of many others who have worked so productively in this area of investigation. The importance of our publication is that it helped demonstrate the usefulness of disaggregation studies in the analysis of organogenesis."

1. Holtfreter S. Gewebeaffinität, ein Mittel der embryonalen Formbildung. *Arch. Exp. Zellf.* **23**:169-209, 1939.

2. Townes P L. Effects of proteolytic enzymes on the fertilization membrane and jelly layers of the amphibian embryo. *Exp. Cell Res.* **4**:96-101, 1953.