tle in my collection continued to excrete this organism for 3 years while remaining in apparent good health. An observer in the south of France has informed me that *Testudo hermanni* is also fond of the more highly flavored scraps which spill from the garbage bin. Though the evidence is scanty I have not discovered any mention of coprophagy as a habit of the other members of the genus *Testudo*.

J. A. BOYCOTT
Public Health Laboratory,
Taunton, England

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3 May 1962

Mechanism of Tissue Reconstruction by Dissociated Cells, II: Time-Course of Events

Abstract. The details of the process by which cells sort out and reconstruct tissues within aggregates containing two kinds of tissue cells have been correctly predicted from considerations of the kinetic and adhesive properties of such cells. The requisite properties are discreteness, motility, and differential mutual adhesiveness among the types of cells present.

Organs or regions of the body of vertebrate embryos may be dissociated into their component cells, which are then capable of reaggregating and sorting out to reconstruct semblances of the original structure (*1, 2*). In these autosynthetic structures the reconstituted tissues are deployed in their normal mutual histological relationships. Such organization usually involves the formation of discrete inner and outer tissues.

In a previous paper (*3*) it was shown that individual cells of the prospective internal tissue do not migrate in a directed fashion toward the center of an aggregate. There did appear to be selection against the residence of such cells at the very surface, however. It was concluded that sorting out must proceed in a manner analogous to that in which a dispersion of mutually immiscible liquids "breaks." In such a dispersion the liquid of lower surface tension (that is, lower molecular cohesiveness or mutual attraction) quickly occupies the surface of the liquid body, during and after which the droplets of the liquid of higher surface tension progressively coalesce to produce a decreasing number of increasingly large islands in the interior. Thus external (continuous) and internal (discontinuous) phases are established. The behavior of such a system is due to its possession of three properties: (i) the two phases are composed of units which are discrete; (ii) the units are mobile; (iii) the different kinds of units are differentially cohesive and adhesive. The first two of these properties are of course known to be characteristic of most cells; but differential mutual adhesiveness, while known for certain kinds of cells, is not established as of general applicability. If sorting-out indeed depends upon differential mutual adhesiveness among the cells in a mixed population, the time-course of events which characterize the process must conform with that given above with reference to dispersions.

Figure 1 shows the sequence of events in the sorting out of chick embryonic heart cells from chick embryonic retinal cells. By virtue of a staining reaction for glycogen (*4*), which they alone contain, the heart cells, derived from 5-day embryos, are distinguishable from the retinal cells, derived from 7-day embryos. Techniques are described elsewhere (*3*). The first event in sorting out is the withdrawal of heart cells from the surfaces of the aggregates. Accompanying this is an initial clustering of heart cells in innumerable foci throughout the interior of each aggregate. These heart foci continue to encounter and fuse with one another, progressively building up one or more coherent, internal masses of heart tissue, the number of which reflects the proportion of heart cells in the population. Townes and Holtfreter have previously described the same sequence of events with amphibian neurula chordamesoderm and endoderm (*2*).

An alternative explanation of the sorting-out phenomenon has been advanced by Curtis (*5*), who suggests that cells of different types undergo certain surface changes at different times after their dissociation. These changes would be such that cells which had experienced them would be trapped by contact either with the surface of an aggregate or with other cells already so trapped. Thus cells of the first type to experience the change would be trapped initially at the surface and then in sequential layers beneath it, leaving those of the type which experiences the change later to be trapped in the

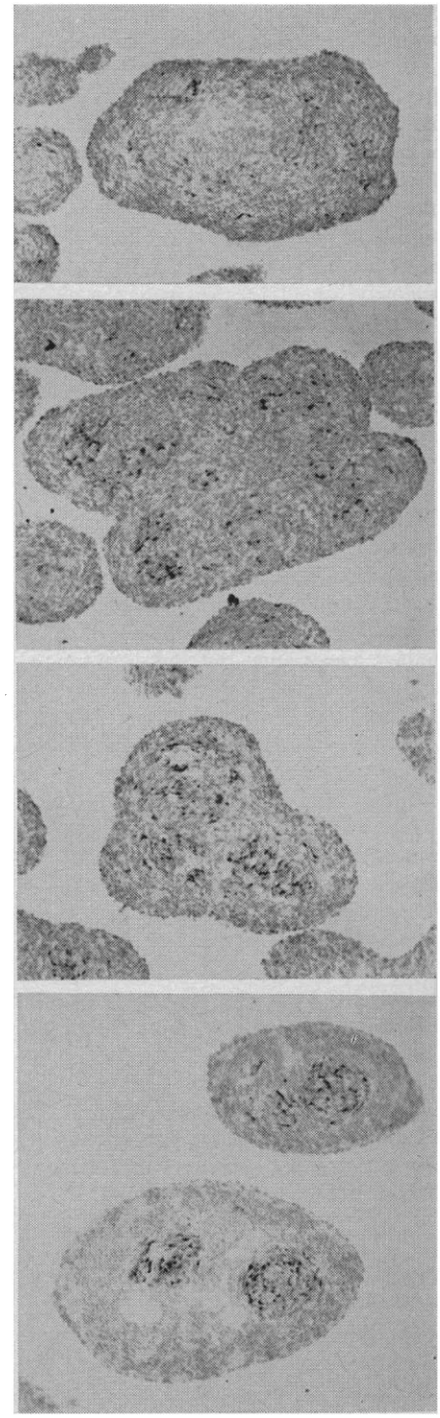

Fig. 1. Sections through aggregates containing chick embryonic heart (darkly stained) and retinal cells fixed at (top to bottom) 17, 24, 31, and 66 hours of incubation at 37°C, showing the process of sorting out (× 127).

center of the aggregate. The operation of this mechanism would produce a herding of the cells of the potentially internal phase, in a centripetal wave, progressively deeper into the aggregates until completion of the separation between the two phases. The actual process of sorting out, as observed, in fact bears no resemblance to that predicted by the "timing" hypothesis.

In the demonstrated absence of directed migration or of a "timing" mechanism, the events described here point strongly toward the action of differential mutual cellular adhesiveness which, acting in a system the units of which are both motile and discrete, is by itself capable of bringing about a separation of the phases in the precise manner and mutual orientation which have been observed. Heart cells must cohere more strongly than retinal cells. The implications with respect to the mechanisms of normal histo- and organogenesis are clear (*6*).

MALCOLM S. STEINBERG
Department of Biology, Johns Hopkins University, Baltimore 18, Maryland

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3 July 1962

Utilization of Nitrogen Compounds by Unicellular Algae

Abstract. Eight Chlorophyta employ urea as a sole nitrogen source; five utilize uric acid and xanthine. The Cyanophyta, Rhodophyta, and Euglenophyta studied do not grow on these three nitrogen sources, although *Anacystis nidulans* decomposes uric acid to allantoin. None of the algae tested utilized either allantoin or creatinine.

The first question to be answered in a consideration of nitrogen metabolism concerns the nitrogenous substances that can be assimilated. A study of the ability of several unicellular algae to utilize the organic nitrogen compounds uric acid, urea, allantoin, xanthine, and creatinine was undertaken.

Nine algae of known taxonomic position and three new isolates were examined. Two of the isolates belong to the genus *Chlorella*. The third is a *Scenedesmus*.

Table 1. Utilization of nitrogen compounds by unicellular algae.

	Urea	Uric Acid	Xanthine	Allantoin	Creatinine	KNO_3	NH_4Cl
Chlorella pyrenoidosa	+	+	+	−	−	+	+
Chlorella vulgaris	+	+	+	−	−	+	+
Scenedesmus obliquus	+	+	+	−	−	+	+
Chlamydomonas reinhardi	+	+	+	−	−	+	+
Asterococcus superbus	+	−		−	−	±	+
Anacystis nidulans	−	*		−	−	+	+
Synechococcus cedrorum	−	−		−	−	+	+
Porphyridium cruentum	−	−		−	−	+	+
Euglena gracilis	−	−		−	−	−	+
Scenedesmus sp	+	+	+	−	−	+	+
Chlorella sp	+	−		−	−	+	+
Chlorella sp	+	−		−	−	+	+

* Decomposes uric acid but does not utilize it for growth.

Four completely defined culture media were employed: Chlorophyta medium (*1*), Cyanophyta medium (*2*), Euglenophyta medium (*3*), and artificial sea water medium (*4*). Uric acid, urea, allantoin, xanthine, creatinine, potassium nitrate, and ammonium chloride were individually substituted for the nitrogen compounds of the original media. The nitrogen concentration was kept between 0.07 and 0.1 g of nitrogen per liter with two exceptions. Uric acid was added at 0.02 g of nitrogen per liter because of its low solubility in water. Allantoin, an optically active compound, was added at 0.2 g of nitrogen per liter. The concentration of all essential elements was maintained during the substitution of the nitrogen compounds. The *p*H did not change appreciably.

Experiments performed to determine whether the organic compounds are toxic to algae in the amounts used gave negative results. In sterilizing the media, the inorganic compounds were autoclaved. The organic components were filtered through a Pyrex ultrafine sintered glass filter (pore diameter, 0.9 to 1.4 μ) or a particle membrane filter (pore diameter, 0.4 μ) and then added to the autoclaved inorganic medium.

Two different culture units were used to grow the algae. The shake table consisted of fluorescent lights placed under a movable Lucite table top which held 16 modified Roux culture flasks. The table moved back and forth a distance of 6.5 cm at the speed of 120 complete shakes per minute. The rolling tubes were 4 cm in diameter and 40 cm long; they rotated at 60 to 120 rev/min (*5*). Compressed air with 0.03 percent carbon dioxide was passed through the culture vessels at a rate of 50 ml/min. Cultures were sampled by inserting a syringe through a rubber stopper on the flask.

Nitrogen utilization was determined by a standardized procedure. An algal inoculum was added to eight culture flasks containing seven different nitrogen compounds plus a nitrogen-free control. Each day, starting with the time of inoculation, a sample was withdrawn from each flask; 0.2 ml was streaked onto a petri dish containing Difco antibiotic medium 3, placed in an incubator at 37°C, and checked visually 24 and 48 hours later for bacterial and mold contamination. Three milliliters of the algal sample was then centrifuged for 5 minutes. The *p*H of the supernatant was measured to determine whether the hydrogen-ion concentration had remained in a physiologically desirable range. Uric acid, xanthine, and creatinine utilization was determined by ultraviolet spectrophotometry. These three compounds have absorption peaks at 290 nm, at 267 nm, and at 235 nm, respectively (1 nanometer = 1 millimicron). Allantoin was determined by paper chromatography. Allantoin gives a red violet spot when sprayed with mercuric acetate and diphenylcarbazone (*6*). Growth was measured by determining the chlorophyll concentration of the algal sediment. The optical density of an 80 percent ethanol extract of the algae was measured at 665 nm, the chlorophyll *a* peak.

Experimental results indicate that there is a wide variation among the algae tested with regard to the nitrogen compounds that they can utilize (Table 1). The Chlorophyta are far superior to any of the other algal phyla tested. All eight utilize urea; five utilize uric acid and the same five utilize xanthine. The Cyanophyta, Rhodophyta, and