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*).

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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

Euglenophyta tested are incapable of utilizing these nitrogen compounds. The one exception, *Anacystis nidulans*, decomposes uric acid to allantoin but is incapable of further degradation. The latter organisms were not grown on xanthine. None of the algae tested utilizes either allantoin or creatinine. All but one of the phyla tested utilize ammonia and nitrate.

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Interaction of Olfactory and Other Environmental Stimuli on Implantation in the Deer Mouse

Abstract. Recently inseminated female deer mice were exposed to changes in physical environment, including size of available space, newness of environment, and a stud or strange male in order to test the hypothesis that a strange male decreases the incidence of pregnancy in recently inseminated females (Bruce effect). The data confirm the Bruce effect but also indicate that changes in physical environment produced great effects on implantation in recently inseminated females.

A number of recent publications by Bruce and her colleagues (*1*) have presented evidence for the role of olfactory stimuli in the regulation of pregnancy in the mouse. The significance of these findings is so apparent that the term "exocrinology" has been proposed for this new area of biology.

The purpose of our study was to test the "Bruce effect" in a wild, non-inbred species in order to determine the incidence of this phenomenon in species other than the laboratory mouse. Experiments were also conducted to determine whether the effect could be modified by factors other than olfactory stimuli. Consequently, these experiments examined the possible effect of changes in the physical environment and whether such changes led to behavioral-psychological disturbances which, accompanied by olfactory stimuli, might lead to failure of implantation.

The species chosen for this investigation was the deer mouse (*Peromyscus maniculatus bairdii*). This subspecies has been recently utilized in a number of comparative endocrine studies (*2*), behavioral experiments (*3*), and anatomical as well as brain studies (*4*).

Subjects used were virgin females of 45 to 60 days of age. All females were paired with a male, hereafter referred to as the stud male, in a 12- by 6- by 6-inch cage and tested for copulation by daily vaginal smears. When presence of sperm was confirmed, the male was removed and the female was isolated for 24 hours in the original cage. After the isolation period, the females were subjected to one of several conditions in which experimental variables were: presence or absence of strange or stud male, freedom or restriction of the male, and size of cage. Exposure to males in all cases was for 24 hours. All females were autopsied 7 days after insemination to determine pregnancy. Implantation occurs between 4.5 and 6 days after mating. Sizes of cages used were: 12 by 6 by 6 inches, 12 by 18 by 6 inches, and 22 by 36 by 10 inches. In the smallest cage (12 by 6 by 6 inches), males were restricted in a 4- by 2- by 2-inch wire box, but in the two larger cages males were not restricted and two sets of food hoppers, water bottles, and nest boxes were present. Twenty females were used for each group.

Our data indicate that as the size of cage increased the incidence of pregnancy in the group with no male decreased ($p = < .05$). In the group of females without a male, the incidence of pregnancy declined from 90 percent in the smallest cage to 30 percent in the largest (Table 1). Although the presence of the stud male resulted in a 30-percent reduction in pregnancy among mice housed in the smallest cage, it produced an approximate 66-percent increase in the largest cage when compared to their respective control groups. The presence of a strange male of the same species resulted in an approximate 75-percent decline in incidence of pregnancy in the smaller cages ($p = <.02$), but showed no change in the largest of the three cages.

Table 1. Number of females pregnant on the 7th day after exposure to various treatments (20 females in each group and each treatment). See text for exact size of cages.

Day 2 of treatment	No. pregnant in cage sizes indicated		
	Small	Medium	Large
Isolated	18	12	6
With same or stud male	12	13	10
With strange male	4	3	6
With empty holding cage	12		
Moved to new quarters with empty holding cage	10		

Furthermore, the introduction of an empty holding cage into the female living quarters, and change of the female to new living quarters, resulted in a 30- to 40-percent decrease in the incidence of pregnancy. In short, a change in the physical environment as well as a change in the male resulted in a decrease in the incidence of pregnancy.

That exposure of a recently inseminated female to a strange male results in a decreased implantation of fertilized ova (Bruce effect) is confirmed by the present results. However, the data also indicate that, in this particular species, odor or presence of a strange male is not the only mechanism which operates to bring about failure of implantation. Our data indicate that changes in either physical environment or social environment may result in a failure of implantation (*5*).

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